

An Innovative Positive Pressure Walk-in Tunnel for Minimizing Insect Infestation and Pesticide Use for Growing Fresh Herbs

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Keywords. basil, fresh herbs, infestation, insects, pesticides, positive pressure, walk-in tunnel

Abstract. Infestation with insects disqualifies fresh spice herbs for local and export markets, severely affecting revenue and the grower's reputation. An innovative positive pressure walk-in tunnel, in which air is drawn and filtered via insect screens into an enclosed, pressurized growing structure was designed and tested as a solution for mitigating crop infestation. In four field trials between 2019 and 2024 with sweet basil (*Ocimum basilicum*), the efficacy of the positive pressure tunnel was tested and compared with the current commercially used passive, pesticide-sprayed tunnel. Insect counts were reduced by 81%. Yields were 55% higher than in the passive control, and basil downy mildew (BDM), caused by *Peronospora belbahrii*, was inhibited in two of three trials in the pressurized system without pesticides. Over the 4-year study, chemical pesticide use was reduced by 88% and the number of basil harvests increased by 33%. An economic viability for sweet basil calculated the positive pressure tunnel to be \$22,000 USD/hectare more profitable than the currently used passive tunnels. The research verified that the positive pressure tunnel was effective in reducing insect penetration and minimizing pesticide applications. Growers incorporating this innovative apparatus are expected to produce crops with reduced pesticide exposure or pesticide-free, appealing to customers' desire for high-quality produce.

Insects and plant diseases are major limiting factors in the production of high-quality agricultural produce, which requires the use of chemical pesticides (fungicides and insecticides) to minimize their harmful impact. Agrios (2005) indicated that on a global scale the annual crop loss to plants caused by pests is estimated to range between 20% and 40% of production and plant diseases are responsible for another, ~\$220 billion USD annual loss. Invasive insects are calculated to cause losses of nearly \$70 billion USD (Bradshaw et al. 2016), and global warming catalyzes this harmful impact (Gullino et al. 2021). In 2021, the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) reported that the total amount of pesticide used in agriculture was 3.5 million tons

globally, indicating an 11% increase in pesticide usage in the past decade (FAO 2021). When partitioned into pesticide categories, the data pointed out that since 1990 the use of fungicides/bactericides and insecticides increased by 111% and 44%, respectively (Wanner et al. 2023).

According to information by the European Union Notification System for Plant Health Interception (EUROPHYT 2024 monthly reports) between 2013 and 2018 a total of 397 shipments of spice herbs, flowers, and fruits, exported from Israel to various locations worldwide were deemed unmarketable at the importing countries' entry ports due to presence of quarantine pests, primarily whiteflies (*Bemisia tabaci*) and leafminers (*Liriomyza* spp.). Of those, 32 (8%) shipments were of freshly

harvested sweet basil (*Ocimum basilicum*), 8 (2%) of fresh mint (*Mentha* spp.), 11 (2.8%) of oregano (*Origanum vulgare*), and 3 (0.75%) of other minor spice herbs grown in Israel for export. The disqualifications caused severe economic impact to the local agriculture industry, especially to the fresh spice herb producers, as well as affected growers' reputations. It should be mentioned that this is in spite of the fact that these products were controlled for pests following the Israeli Ministry of Agriculture pest management protocols (Biton and Silverman 2021; Mor and Bitton 2024) using pesticides approved by the Israeli Plant Protection and Inspection Service (PPIS; <https://pesticides.moag.gov.il>). This alarming situation induced a call to take new actions to improve pest management by innovative methods.

The Valley of Springs (the Bet She'an Valley) in Israel is located in the middle of the Jordan Valley and is a part of the Afro-Syrian rift with an elevation between –100 to –250 below sea level. The region is one of the hottest in Israel with high (>40 °C) temperatures in the summer, mild (10 to 20 °C) in the winter, and moderate (20 to 25 °C) in spring and fall. Relative humidity varies between seasons, ranging between 20% to 30% in the summer, 50% to 80% in the winter and 40% to 60% in fall and spring (Maier 2016). Sweet basil and other spice herbs and leafy vegetables for the fresh market are grown in the region and generally in Israel in screen-houses, greenhouses, or walk-in tunnels. The latter predominate by using commercial walk-in tunnels with width dimensions of 6, 9, or 10 m. The walk-in tunnels are constructed with galvanized steel arches and covered with polyethylene, 50-mesh insect nets, or a combination of both. The plants are grown in the local soil, or plastic troughs filled with tuff (volcanic ash). Irrigation and fertilization are applied via drip irrigation (Nitzan et al. 2012), and during the spring and summer, when temperatures rise, the tunnels are covered with dark shading-nets to lower irradiation and temperature. The walk-in tunnels are passive climate control structures lacking heating, lighting, or active ventilation. Although insect-preventing screens, sticky traps (yellow and blue), double door entries, etc., are used regularly, there still is a great need for pest control by chemical pesticides (insecticides and fungicides). As an example, despite the effort to limit insects, chive production at the Valley of Springs was ended as a result of diminishing thrips control (Lebedev et al. 2013), and today sweet basil production requires ~10 applications of pesticides per season to control insects and downy mildew (Biton and Silverman 2021; Nitzan and Goren 2024).

Positive pressure in agriculture growing facilities refers to the transfer of air using a ventilation system via insect screens into an enclosed growing structure. The internal pressure increases the velocity of air exiting the structure through doors, windows, or other outlets to be higher than the flying speed of insects of concern. Hence, preventing flying insects from entering the growing facility. The outlets may be

passive or motorized to control air speed and internal pressure. Evaporative pads can be added for cooling and increased humidity (Mears and Both 2002; Roberts et al. 1995). These systems were reported to limit infestation with insects and reduce pesticide usage (Roberts et al. 1995).

Studies on positive pressure focus on crop production, primarily tomatoes, in greenhouses or semiclosed greenhouses (Dannehl et al. 2014; Mears and Both 2002; Sapounas et al. 2020). In contrast, studies on positive pressure in polyethylene-covered walk-in tunnels with spice herbs are not available. A polyethylene-covered walk-in tunnel is a less rigid structure than a greenhouse; therefore, developing it into a positive pressure growing facility would be challenging. Nonetheless, walk-in tunnels are less expensive structures than greenhouses and are popular among many small growers in Israel. Retrofitting a positive pressure system to walk-in tunnels for pest management is likely to have a big impact to a wide variety of specialty crop growers in Israel and globally. Success could offer an innovative insect management tactic for growers and potentially limit the increasing dependency on chemical pesticides, which are a major limitation for the marketing of freshly harvested spice herbs.

The principal goal of the present research was to provide the spice herbs, primarily the sweet basil production industry in Israel, with a solution for preventing produce interceptions in Europe and the United States due to infestation with quarantine insect pests by modifying a passive walk-in tunnel into a positive pressure growing system. Nonetheless, the attempt was to reduce crop contamination by all insects, regardless of their function, to achieve insect-free plants, which is the requirement for these crops by the customer. The hypothesis tested in the present research was that a positive pressure walk-in tunnel could assist in minimizing insect infestation and pesticide use in the production of fresh sweet basil.

Materials and Methods

Construction of a positive pressure walk-in tunnel. In 2019, two walk-in tunnels of size 6 m wide \times 30 m long \times 3 m high were

constructed. One tunnel was designated to be a positive pressure prototype, and the other a passive control. In 2023, following promising field trial results, two commercial-size walk-in tunnels of size 9 m wide \times 40 m long \times 4 m high were constructed, with one designated to be a positive pressure tunnel, based on the prototype design, and the other a passive control. The design of the commercial-size positive pressure tunnel is presented in Fig. 1. All tunnels were constructed with galvanized steel arches and were covered with polyethylene greenhouse plastic cover (E460135000150W-0; Ginegar Plastic Products Ltd., Kibbutz Ginegar, Israel, <https://ginegar.com>). In the positive pressure tunnels, the two outermost arches were sealed with a 75-mesh (75 holes/square inch with a hole size between 0.18 and 0.212 mm) insect net (Ginegar Plastic Products Ltd.), used as a flying insects preventive screen. The next nearest arch was constructed with an aluminum frame into which industrial fans (model: EF-28", 18,000 m³/h; www.adirom.co.il) were situated. The fans were tilted upward at an angle of 10° to prevent the wind from blowing directly on the growing plants. The positive pressure tunnel prototype was designed with a single fan, and two fans were placed in the commercial-size positive pressure tunnel. The remaining structure was covered with polyethylene, creating a sealed crop production area. The positive pressure was attained by drawing air through the insect net and forcing it into the production area. The forced air was exhausted through passive shutter outlets situated at the opposite end of the tunnel. The design also included a double door entrance (Fig. 1) constructed in all tunnels (pressurized and passive control). In Summer 2023, an evaporative screen was added to the commercial-size positive pressure tunnel and was placed in the inner arch of the two outermost arches and was covered by the insect screen (Fig. 1). Planting in the prototype tunnel was carried out in three 25-m longitudinal beds in local soil. The commercial-size tunnels were built with five 30-m long \times 1-m wide plastic troughs filled with tuff (volcanic ash). The passive control tunnels (prototype and commercial-size) were similar to the positive pressure tunnels in size, shape, length, width, height, roof shape and double door entrance. They lacked the area used for the insect screen and fans, and were enclosed with 75-mesh screens on the side walls. The passive control tunnels were designed with an insect screen on the side walls, representing the current state of the art used by the industry, which the positive pressure tunnel, developed in the present research, is expected to replace. Planting in the passive control tunnels was similar in soil or plastic troughs. Drip irrigation was used in all structures.

Study location. The study was carried out at the Valley of Spring Research & Extension Center located at the Eden Experimental Farm (Khavat Eden; 32.466091, 35.486808; <https://maps.app.goo.gl/q8aoxEDAkGwbvoDD7>). The farm is situated at the Beit She'an Valley encompassing an area of \sim 0.8 ha of greenhouses, screenhouses, walk-in tunnels, and field plots. Studies in the farm include field,

vegetable and row crops, greenhouse vegetables, spice herbs, dates, etc. Temperature (°C) and relative humidity (%) were monitored using a HOBO UX100 Temp/RH data logger (www.onsetcomp.com). The data loggers were enclosed inside HOBO solar radiation shield RS3-B for outdoor protection and situated in the center of the walk-in tunnel growing area at a height of 50 cm aboveground.

Experimental design. Four field trials with sweet basil (*O. basilicum*; variety 'Perrie'; Gonda et al. 2020) were conducted between 2019 and 2024 (Table 1). The trials tested differences in insect counts, yield, and BDM, caused by *P. belbahrii*, between the positive pressure tunnel and the passive, nonpressurized pesticide-sprayed control tunnel. Each trial included one pressurized and one passive, pesticide-sprayed control tunnel. Trial 1 was carried out in Fall 2019 in the 6-m \times 30-m tunnels. Sweet basil was planted into local soil in three 1-m wide \times 25-m long beds with four planting rows. Trials 2, 3, and 4 were carried out in Spring 2023, Fall 2023, and Spring 2024, respectively, in the commercial-size 9-m \times 40-m tunnels, where sweet basil was planted into five 1-m \times 30-m tuff-filled troughs in four planting rows. The plants were purchased from a local nursery (<https://www.hishtil.com>) and were planted at the six to eight true leaves stage (\sim 1-month old plants). Planting density was 20 plants/m² following the local commercial protocol. The plants were drip-irrigated at rates of 25,000 and 30,000 L ha⁻¹ in the fall and spring, respectively; and were fertigated at a rate of 1500 L ha⁻¹ with Mor 3.3-2-6 (N-P-K + microelements; Deshanim, <https://iclgrowingsolutions.com>) following the local commercial practices (Nitzan and Goren 2024).

Fungicides and insecticides. Insects and diseases were managed using insecticides and fungicides registered in Israel for sweet basil. Applications were carried out according to label recommendations approved by the Israeli PPIS (<https://pesticides.moag.gov.il>), and according to the Ministry of Agriculture Extension Services guidelines (Biton and Silverman 2021; Mor and Bitton 2024). The threshold for exported sweet basil is lack of quarantine insects. As well, lack of any insect is expected by the customer. Therefore, the threshold is "zero" insects (Biton and Silverman 2021). Application of chemical control in the passive control tunnel followed a prophylactic protocol as used by growers in the region. Application in the positive pressure tunnel, which is intended to be a preventive insect and pest management growing system, followed a responsive/curative protocol. All information about the pesticides used and dates of applications are provided in Supplemental Table 1. The following fungicides and rates were used: Acrobat® SC (a.i. dimethomorph 500 g L⁻¹; BASF Crop Solutions, Ludwigshafen/Rhein, Germany, www.basf.com; rate: 2 kg ha⁻¹); Cabrio® DM (a.i. dimethomorph 72 g L⁻¹, pyraclostrobin 40 g L⁻¹; BASF Crop Solutions; rate: 2 L ha⁻¹); Canon® (a.i. potassium phosphite 780 g L⁻¹; Luxembourg Industries Ltd., Tel Aviv, Israel,

Received for publication 11 Dec 2024. Accepted for publication 23 Dec 2024.

Published online 18 Feb 2025.

We thank Mr. Shimon Lahiani and Mr. Mahmud Zoabi for technical support. The research was funded through the Israeli Spice Herb Commission, The Israeli Ministry of Agriculture - Extension and Education Grants, The Israeli Ministry of Agriculture - Chief Scientist Extension grant support, and The Israeli Ministry of Justice - Inheritance Affairs. The funders were not involved at any stage of the research with study design, collection, analysis and interpretation of data, writing of the report, or the decision to submit the article for publication.

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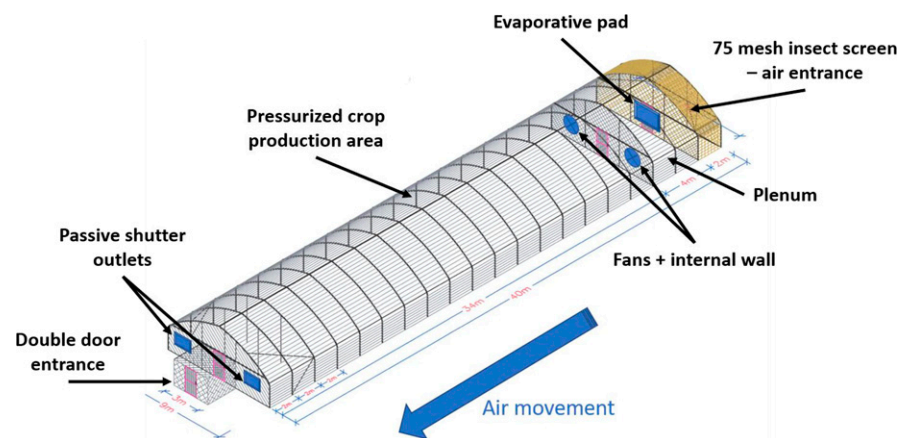


Fig. 1. A diagram of the 9-m wide \times 40-m long \times 4-m high polyethylene-covered, arch-framed, positive pressure commercial-size walk-in tunnel as constructed and tested at the Valley of Springs Research & Extension Center, Israel. The commercial-size tunnel was constructed following the design examined with a 6-m \times 30-m prototype tunnel, which included a single fan.

luxembourg.co.il; rate: 35 L \cdot ha $^{-1}$). The following insecticides were used: Biomactin[®] (a.i. abamectin 18 g \cdot L $^{-1}$; Tapazol Chemical Industries Ltd., Beit Shemesh, Israel, tapazol.co.il; rate: 500 mL \cdot ha $^{-1}$); Perfect[®] (a.i. emamectin benzoate 19.2 g \cdot L $^{-1}$; Tapazol Chemical Industries Ltd.; rate: 600 mL \cdot ha $^{-1}$ and 1% v/v for sweet basil and green onion, respectively); Metazon[®] (a.i. metaldehyde 5%; Rimi Chemicals Ltd., Petah Tikva, Israel; rate: 20 kg \cdot ha $^{-1}$); Movento[®] 100 (a.i. spirotetramat 100 g \cdot L $^{-1}$; Lidor Elements Ltd., Ramat HaSharon, Israel, www.lidorr.com; rate: 500 mL \cdot ha $^{-1}$); NeemGard[®] (a.i. neem oil 70%; ADAMA Ltd., Airport City, Israel, www.adama.com; rate: 1% v/v); Proclaim[®] (a.i. emamectin benzoate 19.2 g \cdot L $^{-1}$; ADAMA Ltd.; rate: 600 mL \cdot ha $^{-1}$) and Vertimec[®] 18EC (a.i. abamectin 18 g \cdot L $^{-1}$; ADAMA Ltd.; rate: 500 mL \cdot ha $^{-1}$). Weeds were removed manually without the use of herbicides. Every summer, between June and September the growing media (local soil or tuff) was sanitized by soil solarization.

Data Collection

Insect quantification. Whiteflies, leafminers, and other insects were monitored on both plants and yellow sticky traps. The yellow sticky traps (n = 8 to 10) were placed in each tunnel (pressure and control) at equal distances of 4 m along the longitudinal walls and

the number of flying insects were counted at the end of each trial. Plant infestation by whiteflies, leafminers, or other insects was monitored before harvest. Five plants from 10 randomly selected locations within a tunnel (n = 50 plant) were visually inspected and the number of insects was recorded.

BDM quantification. BDM, which is currently the primary and most destructive disease of commercially produced sweet basil in Israel, was the disease of interest. Downy mildew symptoms were monitored throughout the growing season. Disease incidence was scored visually, recording the proportion (%) of plants with downy mildew symptoms. Disease severity was scored visually on a 0 to 6 ordinal category scale, as follows: 0 = healthy plants, 1 = 1% to 5% of leaves chlorotic, 2 = 6% to 10% of leaves chlorotic with sparse sporulation on the abaxial side of the inflicted leaf area, 3 = 11% to 25% of leaves chlorotic with some necrosis and profuse sporulation on the abaxial side of the inflicted leaf area, 4 = 26% to 50% of leaves necrotic with profuse sporulation on the abaxial side of the inflicted leaf area, 5 = > 50% of leaves necrotic with profuse sporulation on the abaxial side of the inflicted leaf area, 6 = dead plant (Nitzan and Goren 2024). Disease severity and incidence were used to calculate a disease severity index (DSI) using the formula: $DSI = \sum (X_i \times P_i) / 100$; where X_i

represents the disease severity and P_i the incidence (%) of diseased plants within the disease severity category. The DSI ranged between 0 = no disease and 6 = entire plot with dead plants. The area under the disease progress curve (AUDPC) was calculated using the formula: $AUDPC = \sum_{i=1}^{n-1} [(y_i + y_{i+1}) / 2] \times (t_{i+1} - t_i)$; where y_i and y_{i+1} are respectively the DSI scores at t_i and t_{i+1} (Campbell and Madden 1990; Madden et al. 2007).

Yield. Fresh basil was harvested at ~25- to 30-d intervals during the trials and at each trial one to three harvests were carried out (Table 1). The harvest was executed by a local grower/exporter to adhere to commercial marketing standards, where ~20 to 25 cm of fresh foliage is removed. At each harvest date, a 1 m \times 1 m square frame was randomly placed at 10 locations in the walk-in tunnels, sampling 20 plants at each location. The mean yield per harvest (g \cdot m $^{-2}$) and the combined (accumulated) seasonal yield (g \cdot m $^{-2}$) were calculated.

Statistical analysis. The experimental design included only a single replicated walk-in tunnel per treatment at each trial (growing season): i) a positive pressure tunnel and ii) a passive, pesticide-sprayed control. Because the tunnels were not replicated within each trial, the trials/growing seasons (Fall 2019, Spring 2023, Fall 2023, and Spring 2024) were used as replications/blocks, and the analysis of variance (ANOVA) was carried out as a randomized complete block design with a one-way structure (tunnel: pressurized or passive control). Insect and disease inspection and sampling on the plants were carried out at random within each tunnel, and counts on the yellow sticky traps were recorded. Insect counts were transformed using $\sqrt[3]{y_i + 0.5}$, where y_i represents the number of insects. BDM DSI scores were used to calculate the AUDPC and ANOVA was employed on the ranked data following the Kruskal-Wallis procedure as AUDPC values arose from an ordinal scale. Tukey's honestly significant difference was used as post hoc test for mean separation. Data are presented as average \pm standard error or 95% confidence interval. Back-transformations were used where relevant (Zar 1996). All statistical analyses were performed at $\alpha = 0.05$ in JMP 18 (JMP Statistical Discovery LLC, www.jmp.com).

Table 1. Trial information, number of pesticide applications, and basil harvest in four field trials evaluating the potential of the positive pressure walk-in tunnel for minimizing insect counts and pesticide use.

Trial ⁱ	Year / Season ⁱⁱ	Initiation	Termination	Duration (days)	No. of pesticide sprays ⁱⁱⁱ		No. of harvests per season	
					Passive	Pressure	Passive	Pressure
1	2019 / Fall	27.11.2019	11.03.2020	105	6	0	1	3
2	2023 / Spring	23.02.2023	10.05.2023	76	3	0	1	2
3	2023 / Fall	11.10.2023	14.01.2024	95	10	3	2	2
4	2024 / Spring	16.04.2024	25.06.2024	70	5	0	2	2
4 trials summary					24	3	6	9

ⁱ All trials were carried out at the Valley of Springs Research and Extension Center at Eden Experimental Farm.

ⁱⁱ The sweet basil cv. Perrie was used in all trials.

ⁱⁱⁱ Pesticides = fungicides and insecticides.

Table 2. Temperature and relative humidity recorded in the passive and positive pressure tunnels during the research.

Trial	Year / Season	Temp (°C) ⁱ		No. of hours T ≥ 30 °C ⁱⁱ		RH (%) ⁱ		No. of hours RH ≥ 85% ⁱⁱ	
		Pressure	Passive	Pressure	Passive	Pressure	Passive	Pressure	Passive
1	2019 / Fall	23.1 ± 0.08 a ⁱⁱⁱ	20.3 ± 0.06 b	114	77	80.2 ± 0.08 a	68.2 ± 0.1 b	224	62
2	2023 / Spring	22.7 ± 0.1 a	21.9 ± 0.08 b	322	260	79.5 ± 0.16 a	76.9 ± 0.2 b	643	654
3	2023 / Fall	23.8 ± 0.23	23.4 ± 0.2	199	168	73.1 ± 0.5 b	75.6 ± 0.6 a	332	463
4	2024 / Spring	26.9 ± 0.18 b	27.8 ± 0.17 a	539	569	83.9 ± 0.36 a	72.7 ± 0.46 b	927	502
All seasons summary		25.6 ± 3.7	24.9 ± 4.5	Σ = 1201	Σ = 1102	76.2 ± 7.7	72.4 ± 3.9	Σ = 2130	Σ = 1696

ⁱ Values are daily average ± standard error.ⁱⁱ T = temperature, RH = relative humidity, Σ = sum.ⁱⁱⁱ Different lowercase letters represent statistical differences at α = 0.05.

Results

Pesticide application. Four field trials were performed between 2019 and 2024 to compare the ability of the positive pressure walk-in tunnel to limit insects and minimize chemical control to the passive walk-in tunnel, which is the current state of the art used by the local growers. Sweet basil, which is the leading spice herb in Israel and the region was used. Sweet basil grown in positive pressure was sprayed with pesticides only three times over the duration of the four trials/growing seasons, in contrast to its counterpart grown in the passive control, which received 24 applications (Table 1), as required by the prophylactic plant protection protocol. Therefore, over the entire course of the study (four growing seasons), by using the positive pressure technology chemical pesticide applications were reduced by 88%.

Temperature and relative humidity. The daily average temperatures in the positive pressure tunnel were recorded to be slightly higher ($P < 0.05$) than in the passive control in two of four trials (Table 2). However, across the four trials, the positive pressure tunnel was warmer than the passive control with 99 more hours of temperatures ≥ 30 °C. Daily average relative humidity values in the positive pressure tunnel were recorded to be higher ($P < 0.05$) than in the passive control in three of four trials (Table 2). Across the four trials, the positive pressure tunnel

exhibited 434 h more of relative humidity ≥ 85% compared with the passive control, primarily due to trial 4 in Spring 2024, where 927 h with relative humidity ≥ 85% were recorded.

Yield. Sweet basil grown under positive pressure was harvested nine times in contrast to six times in the passive control, primarily due to the development of BDM. Hence, indicating a 33% increase in the number of sweet basil harvests (Table 1). Across the four trials, sweet basil grown in the positive pressure tunnel yielded an average of 54% more ($P < 0.0001$) fresh yield per harvest and overall per season compared with sweet basil grown in the passive control tunnel (Fig. 2).

Insect infestation. Over the course of the four trials, basil plants infested with whiteflies and/or leafminers were not observed. A single thrips was recorded on sampled basil leaves in trial 3 (Fall 2023); and a single mealy bug and a couple of flies were observed in the positive pressure tunnel at the end of trial 4 (Spring 2024). Insect counts on yellow traps were 81% less ($P < 0.0001$) in the positive pressure tunnel than in the passive control tunnel (Fig. 3A), pointing out that a significantly lower insect load was maintained in the pressurized tunnel without insecticide applications compared with its counterpart. Representative images of yellow sticky traps in the positive pressure tunnel and in the passive, pesticide-sprayed control tunnel, are presented in Fig. 3B and C.

BDM. BDM, caused by *P. belbahrii*, developed through naturally occurring inoculum. In Fall 2019 and Spring 2023, BDM developed only in the passive, pesticide-sprayed control tunnel, but not in the positive pressure tunnel (Fig. 4A). However, in Fall 2023, BDM developed in both tunnels with a more pronounced disease in the positive pressure tunnel in early December (Fig. 4B). Because BDM had not developed before in the positive pressure tunnel, a disease control procedure was applied. The infected foliage was trimmed, and the fungicides Acrobate® and Cabrio® DM were applied, which limited the severity of BDM. Hence, in mid-December, following the intervention protocol in the positive pressure tunnel, levels of disease were reduced to minute severities in both tunnels (Fig. 4B). Applications to prevent BDM in the passive control tunnel followed a prophylactic protocol, which elucidates the low levels of BDM. In contrast, similar with insects, the positive pressure tunnel followed a curative protocol, as this tunnel is intended to act as a protective growing system. Despite the fungicide intervention, during Fall 2023 trial the positive pressure tunnel received three fungicide sprays, compared with 10 fungicide and insecticide sprays in the passive control (Table 1). BDM did not develop in Spring 2024. Images of sweet basil grown disease-free in the positive pressure tunnel or severely infected with BDM in

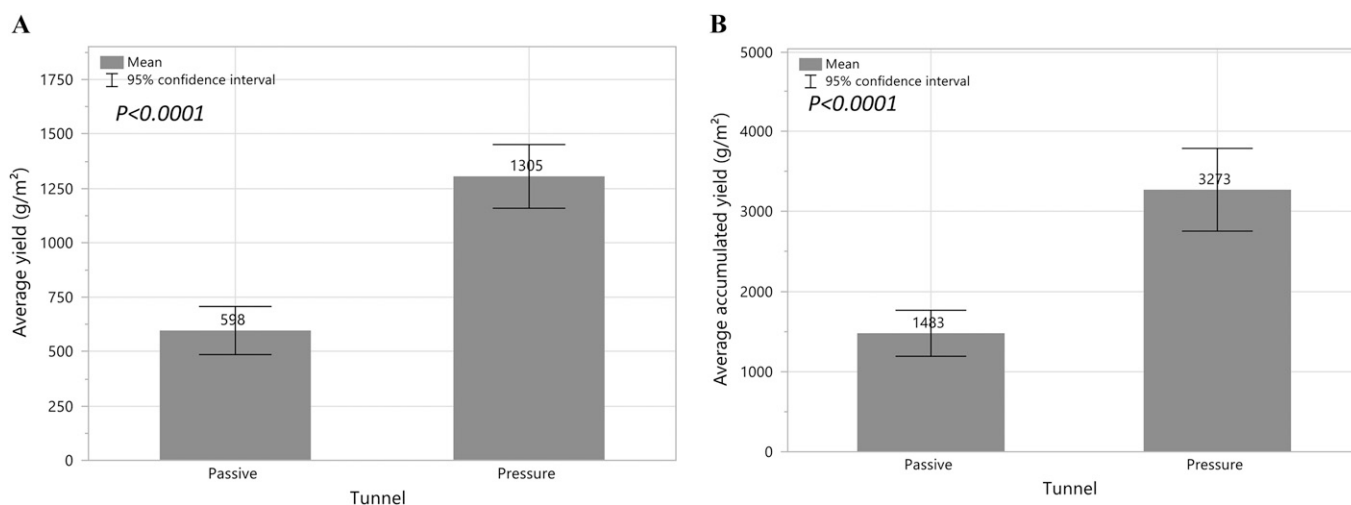


Fig. 2. Average yield per harvest (A) and average accumulated (overall) yield per season (B) recorded in Fall 2019, Spring 2023, Fall 2023, and Spring 2024 to evaluate the positive pressure tunnel as means to minimize insects and pesticide use for sweet basil production. Analysis of variance used each growing season as a replication due to the presence of a single walk-in tunnel for comparison. P value indicates level of significant difference.

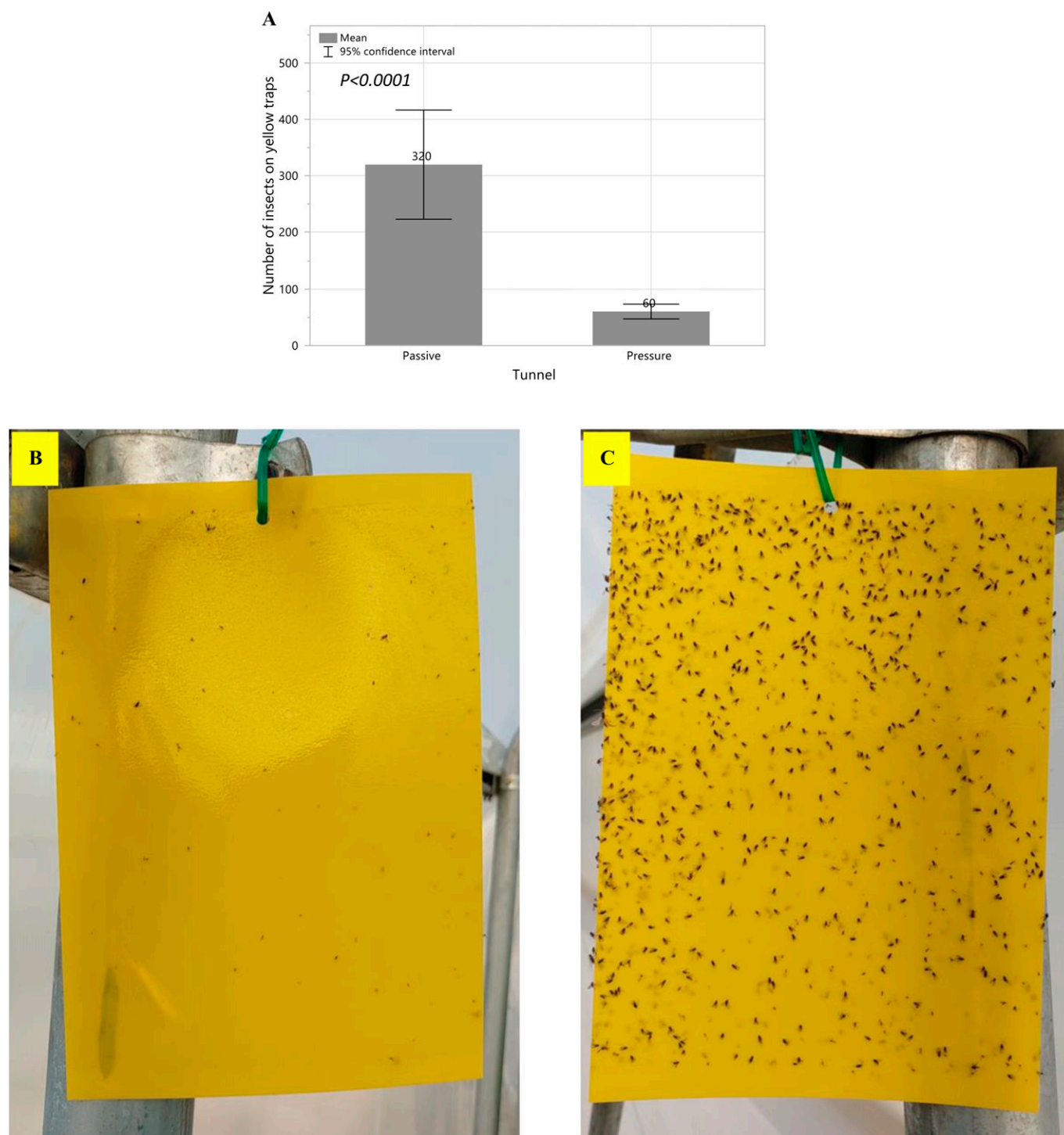


Fig. 3. Average number of insect counts on yellow sticky traps in four field trials (Fall 2019, Spring 2023, Fall 2023, and Spring 2024) (A). Representative images of infested and noninfested yellow traps in the positive pressure tunnel (B) and the passive control tunnel at the end of the Spring 2023 trial. P value indicates level of significant difference.

the passive, pesticide-sprayed control tunnel are presented in Fig. 4C and D.

Discussion and Conclusions

The present study was a public research and development-funded effort to provide commercial sweet basil and spice herbs growers in Israel with a solution for exported produce interceptions in Europe and the United States due to infestation with quarantine insect

pests. An innovative positive pressure walk-in tunnel was developed and tested as a possible future replacement to the currently used passive walk-in tunnels. As the expectation by the customer is for insect- and disease-free spice herbs, primarily sweet basil, the goal was to reduce crop contamination by all insects, regardless of their function and achieve insect- and disease-free plants with limited exposure to chemical pesticides.

Four growing seasons of field trials provided evidence that the positive pressure tunnel reduced insect infestation (pests and others) and BDM severity, while providing similar or better yield and quality than the currently industry-used pesticide-sprayed passive control walk-in tunnel. Consequently, the results of the study indicated that the innovative positive pressure walk-in tunnel outperformed the current state of the art, effectively minimizing insect load and foliar diseases in sweet basil, while

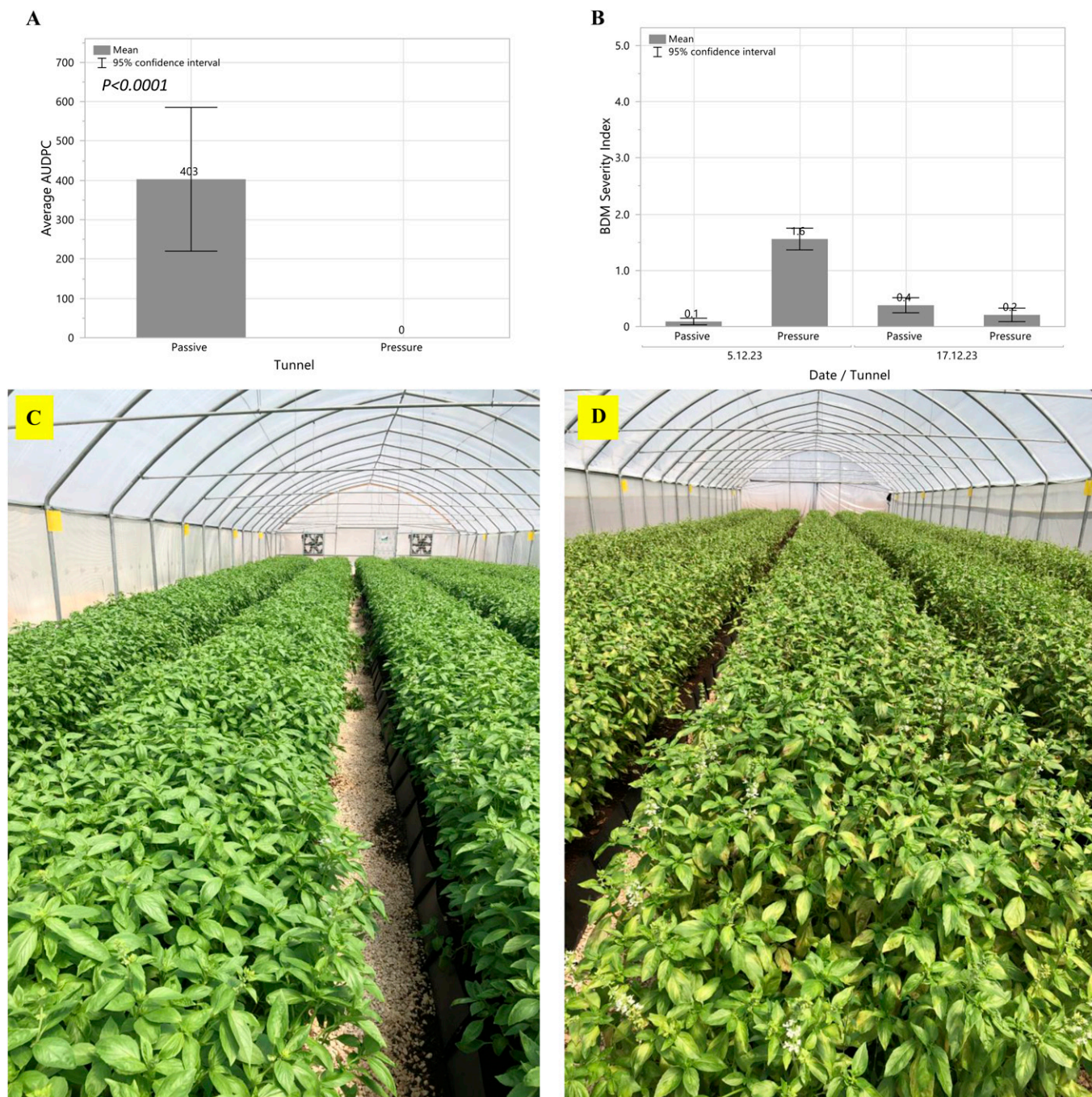


Fig. 4. Average amount of basil downy mildew (BDM) as represented by the area under the disease progress curve (AUDPC) in the Fall 2019 and Spring 2023 trials (A). Descriptive statistical representation of BDM severity in the Fall 2023 trial (B) and representative images of sweet basil grown disease-free in the positive pressure tunnel (C) or severely infected with BDM in the passive, pesticide-sprayed control tunnel (D) during the Spring 2023 trial. P value indicates level of significant difference following analysis of variance.

reducing pesticide use over the entire duration of the study by 88% (Table 1).

Positive pressure greenhouses have been explored and demonstrated to be effective in controlling pests. Roberts et al. (1995) reported that positive pressure reduced pesticide use, pointing out that by carefully monitoring insect counts with sticky traps inside the greenhouse, only localized pesticide applications were required (Mears and Both 2002). Sugiyama et al. (2014) tested control of insects in a greenhouse with positive pressure forced ventilation, using 1- or 0.4-mm mesh screens, indicating that the positive

pressure greenhouse prevented leafminers, was moderately effective in limiting whiteflies, and ineffective for preventing thrips. The positive pressure walk-in tunnel design developed in the present research used a highly dense, 75-mesh screen, which is ~ 0.15 mm in mesh size and is intended to prevent thrips penetration, which could explain the discrepancies between the two studies.

The positive pressure walk-in tunnel was not designed with the purpose of controlling BDM, caused by *P. belbahrii*. Yet, in two of three trials with sweet basil, when BDM developed in the passive control tunnel, it was

suppressed in the positive pressure tunnel without fungicides (Fig. 4A, C, and D). In Fall 2023, BDM developed in the positive pressure tunnel (Fig. 4B) and was controlled with only three fungicide applications compared with seven in the passive control. Consequently, minimizing fungicide usage by 57%. A plausible explanation for the suppression of BDM is that the positive pressure tunnel was warmer ($\geq 30^{\circ}\text{C}$) than the passive control in the discussed trials (Table 2). This observation agrees with Cohen and Rubin (2015), who indicated that daytime solar heating reduced the viability and infectivity of *P. belbahrii* spores

resulting in suppression of BDM. Furthermore, the fans in the tunnel operate continuously to maintain the positive pressure, creating constant air movement. Hence, congruent with the finding of Cohen and Ben Naim (2016), who reported nocturnal fanning to assist with BDM control. BDM is a major constraint for commercial production of sweet basil in Israel, and disease control is dependent on a limited number of fungicides, restricted by low maximum residue levels (Biton and Silverman 2021). In light of these results, the positive pressure tunnel is likely to provide growers with added values beyond insect control.

During the study it became clear that the positive pressure tunnel is not a sterile growing environment or without challenges. To make the tunnel commercially operative, additional research is required to customize or adjust plant protection protocols similar to the intervention procedure executed against BDM in the Fall 2023 trial (Table 2, Fig. 4B). The tunnel does not prevent penetration of nonflying insects, such as mealy bugs that are attached and transferred by ants (Jahn et al. 2003; Puspitasari et al. 2023) or flies and gnats associated with organic matter in growth media that may arrive with seedlings from the nursery. It is suggested to control weeds or soilborne pathogens by employing pre-season soil solarization and in-season manual weeding inside and outside the structure. Before planting, the plants should be inspected and treated for insect eggs or larvae and fungicide application should be considered to limit the probability of latent infection by plant disease causing phytopathogens. Being an enclosed growing system, good sanitation should be kept when entering the tunnel to limit the likelihood of insect and pathogen carryover from fields or other growing facilities on workers' boots and clothes, as infestation within the tunnel would require immediate and thorough pesticide intervention. The insect screens should be kept clean to prevent clogging with dust or sand, which may limit air flow (Mears and Both 2002), and the polyethylene cover is to be scouted for holes and tears on a regular basis. Furthermore, to maintain pressure, the fans must operate continuously. A malfunction, stopping the fans at night or during the heat of the day, may severely damage the crop, necessitating maintenance, an alarm system, and a power back-up. Furthermore, despite the active ventilation, the positive pressure tunnel was warmer than its counterpart, which could prove problematic with heat-sensitive crops such as tomato (*Solanum lycopersicum*) (Alsamir et al. 2021), a crop of interest for future research. Further research is essential to develop crop-specific agro-techniques for use with the positive pressure tunnel commercially.

An economic viability calculation was carried out for sweet basil cultivation as a model crop in the positive pressure tunnels using Israeli Shekel (ILS) as currency and dunam (0.1 ha) as the unit of growing area. The required investments for implementing the positive pressure tunnel technology includes a 75-mesh net screen, an electrical panel, a climate control system, a humid mattress, two

fans, and a generator. The investment cost per dunam, with an additional 30% installation, is estimated at 90,000 ILS (~\$24,270 USD), with a capital return of ~16,000 ILS (~\$4320 USD). The positive pressure walk-in tunnel annual expenses are estimated at 5500 ILS (~\$1500 USD). Using the positive pressure tunnel technology for sweet basil cultivation has a significant advantage by providing a high-quality, insect-free yield compared with the conventional cultivation passive tunnels. This allows for a maximum marketed yield of 6 t per dunam, compared with 2.5 to 3 marketed metric tons per dunam using the currently used passive walk-in tunnel due to insect and disease problems. Hence, the additional marketed yield increases revenue by ~48,000 ILS (~\$13,000 USD) per dunam. Subtracting harvesting, sorting, and packaging costs of ~22,000 ILS (~\$6000 USD) per dunam, the net additional revenue after deducting additional labor and packaging costs is estimated at 26,000 ILS (~\$7000 USD) per dunam. Furthermore, the positive pressure technology reduces pest control costs to about one-third of the usual amount, savings of nearly 3400 ILS (~\$917 USD) per dunam in pest control expenses. In summary, the investment in the positive pressure tunnel technology and its usage adds an annual profit of ~8000 ILS per dunam for sweet basil cultivation compared with the passive walk-in tunnel cultivation without the technology, which is ~\$22,000 USD per hectare.

The present study is intended to continue with model farms for sweet basil and tomato at commercial growers' sites, which will allow an in-depth economic evaluation of the positive pressure walk-in tunnel benefits under commercial growing environments. Nonetheless, the key added value is the substantial reduction in chemical pesticide usage, which increases the marketing potential of the grown crop. As a result, the growers may sell their produce at higher market prices (pesticide-free, insect-free, etc.) that could justify the investment needed for the construction and maintenance of the positive pressure system. Needless to say, the reduction in chemical applications benefits public health and increases environment and ecological safety, both non-measurable within the scope of the current study. In addition, tomato is a staple vegetable crop in Israel, grown in greenhouses. It is prone to many foliar diseases and insect pests, some vectors of notorious viruses. These limit tomato production and quality to the point in which the state of Israel is required to import tomatoes to provide the public's demand. With this in mind, future research of the positive pressure walk-in tunnel is planned to focus on greenhouse-grown tomato. A challenging barrier to resolve is heat management, as tomatoes are heat sensitive. Nevertheless, once demonstrating that the positive pressure walk-in tunnel outperforms the currently used passive tunnel under model farm conditions, and together with financial support from the Ministry of Agriculture for construction of new tunnels or retrofitting passive ones, the industry is

expected to adopt the positive pressure walk-in tunnel technology.

The modern intensive agriculture production relies on synthetic pesticides as the foundation for effective crop protection, despite the increasing evidence of toxicity to human health, increased pest resistance and resurgence, while impairing natural enemies and pollinators (Abubakar et al. 2022; Bakker et al. 2020; Jacquet et al. 2022; Jepson et al. 2020). Public policies are urging toward reduction in pesticide use and more sustainable and ecological agriculture systems (Jacquet et al. 2022; Nicolopoulou-Stamati et al. 2016). The innovative positive pressure walk-in tunnel designed and developed in the present research can provide a satisfactory solution for both conventional and organic growers. The simple and low-cost design is an advantage over highly sophisticated semiclosed greenhouses. Growers who decide to incorporate the positive pressure walk-in tunnel system into their farms are likely to reduce the reliance on pesticides, harvest crops with reduced exposure or even pesticide-free, and provide the public with the high-quality produce it desires. For maintaining the positive pressure within the tunnel, future research is expected to explore solar and wind power as sources of green and renewable energy.

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