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Smart Surveillance of Tomato Viral Diseases: A Decentralized Point-of-Care-Based Diagnostic Network to Enhance Sustainable and Resilient Crop Protection

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Abstract

Plant viral diseases threaten the tomato agricultural industry. A smart decentralized diagnostic network was realized across the main Sicilian tomato-producing provinces for real-time detection/monitoring of *Begomovirus solanumdelhiense* (tomato leaf curl New Delhi virus—ToLCNDV), transmitted by *Bemisia tabaci*, *Tobamovirus fructirugosum* (tomato brown rugose fruit virus—ToBRFV), *Orthotospovirus tomatomaculae* (tomato spotted wilt virus—TSWV) and *Amalgavirus lycopersici* (southern tomato virus—STV). The network deployed smart portable thermocyclers and ready-to-use molecular diagnostic kits (real-time RT-LAMP, RT-qPCR). Data were remotely analyzed and in situ application of the developed kits was evaluated. Results revealed widespread STV infection (>70%) across all provinces, a variable ToBRFV presence with higher incidence in Ragusa (65%) and Siracusa (55.6%) provinces, ToLCNDV mainly concentrated in Siracusa (61.4%) and Trapani (60.2%) provinces, and localized TSWV outbreaks. ToLCNDV detection in *Bemisia tabaci* MED specimens confirmed the vector's role in field transmission (up to 100% incidence). Performance comparison between laboratory and point-of-care conditions showed comparable accuracy, specificity, robustness, and rapid, cost-effective virus detection/monitoring. This diagnostic network enhances early diagnosis and timely phytosanitary interventions in tomato crops. The system supports integrated management strategies by reducing diagnostic delays and improving outbreak containment, control measures application and agroecosystem stability.

Keywords: sustainable agriculture; tomato diseases; insect vectors; remote detection; integrated management; real-time LAMP; real-time PCR

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1. Introduction

Native to South and Central America, tomato (*Solanum lycopersicum* L.) was introduced to Europe by Spanish explorers in the sixteenth century, where it has been cultivated for

about 400 years, to later spread across the globe [1]. Being used for both fresh-fruit consumption and processed products, it is the second most cultivated vegetable in the world after potato, with a global economic significance [2]. In 2023, the world's tomato production reached approximately 192 million tons (MT) from 5,412,458 million hectares of harvested area globally, with almost 13 MT produced in South Europe and more than 11 MT in North Africa. According to the Food and Agriculture Organization of the United Nations, Italy ranks third on the list of top 10 tomato-producing countries in the Mediterranean basin after Turkey and Egypt, with a total production of 6.02 MT from 90,000 hectares of harvested area [3].

Sicily is the first largest greenhouse tomato-producing region in Italy with 201,126 tons from 3029 hectares of harvested area. Sicilian tomato production is mainly located in the Ragusa Province, realized in about 200,000 hectares that in 2023 provided almost 70% of the total Sicilian tomato production under greenhouses, with 140,000 tons; listed in decreasing order by production, important Sicilian provinces also include Siracusa, Palermo, Caltanissetta, Agrigento, Trapani, Catania and Messina. Moreover, Sicily ranks fifth among the main Italian regions in terms of open field tomato production, after Emilia-Romagna, Puglia, Lombardia and Campania, with 226,353 tons from 12,230 hectares of total harvested area in 2024, of which 154,603 tons were consumed fresh and 71,750 tons were processed [4].

Today, safeguarding food security is at the forefront of global efforts where the main current concern is ensuring both availability and access to food for consumers at reasonable prices. These main objectives were set up within the 39th article of the Treaty on the Functioning of the European Union (TFEU). However, achieving these objectives faces a number of challenges, including pathogens, that have put the agriculture sector under increasing pressure all over the world [5]. The FAO estimates that plant pests and diseases cause up to 40% of yield losses worldwide, with a cost of approximately USD 220 billion annually [6,7]. According to the European Commission (2023), vegetable crop losses worth several billion euros occur annually due to viral diseases and the emergence of new viruses in European fields and greenhouse crops.

Plant viral diseases, being a part of over 200 pests and diseases identified in tomato, pose a major threat to the tomato agricultural industry. The recent most threatening viruses which are responsible for severe diseases outbreaks across the Mediterranean basin, particularly in Sicily, are the begomoviruses (family *Geminiviridae*), such as *Begomovirus solanumdelhiense* (Tomato leaf curl New Delhi virus—ToLCNDV) [8], recently listed by the European and Mediterranean Plant Protection Organization as a quarantine pest in the A2 List [9]. Within the same family, tomato yellow leaf curl disease—TYLCD, caused by *Begomovirus coheni* (tomato yellow leaf curl virus—TYLCV) as well as *Begomovirus solanum-flavusardiniaense* (tomato yellow leaf curl Sardinia virus—TYLCSV) and its recombinants, are undoubtedly among the greatest potential threats to the tomato industry and are listed in the EPPO A2 List too, along with the newly identified virulent tobamovirus known as *Tobamovirus fructirugosum* (tomato brown rugose fruit virus—ToBRFV) [10], recently classified by the EPPO as RNQP (Regulated Non-Quarantine Pest) [11]. ToBRFV, belonging to the *Virgaviridae* family, was first identified in tomato crops grown under greenhouses in Jordan [12]. It is considered to be the most virulent known virus, capable of breaking all tobamovirus resistance genes in tomatoes; several ToBRFV outbreaks have been reported worldwide, primarily by human-assisted spread and possible transmission by the bumblebee *Bombus terrestris* L. (Hymenoptera: Apidae) and the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) [13].

Likewise, *Orthotospovirus tomatomaculae* (tomato spotted wilt virus—TSWV) [14], recently attributed to the *Tospoviridae* family by the International Committee on Taxonomy of

Viruses (ICTV), is one of the most pandemic-related agricultural orthotospoviruses, listed into the EPPO A2 List, causing significant agronomical problems to tomato and other host plants [15]. This virus is transmitted by several species of thrips in a persistent propagative manner [16] and causes serious crop losses worldwide [14].

Also, the *Potexvirus pepini* (Pepino mosaic virus—PepMV), integrated into the EPPO A2 List in September 2012, represents a serious threat to commercial tomato production after having remained insignificant for almost twenty years [17,18]. In addition, the newly emerging *Blunervirus solani*, known as the tomato fruit blotch virus—ToFBV, is worth mentioning and was recently added into the EPPO Alert List in January 2024 [19]. Ultimately, *Amalgavirus lycopersici* (southern tomato virus—STV), a member of the *Amalgaviridae* family, is exclusively transmitted vertically through infected seeds [20]. Although the virus has been reported not to induce visible symptoms on singularly infected tomato plants [21,22], it has been demonstrated to have a synergetic effect when co-infecting tomato plants with other viruses, such as PepMV or cucumber mosaic virus—CMV, which worsens the symptoms [23,24].

In this context, there have been initiatives in recent centuries related to tomato domestication, breeding and population bottleneck, which increased the rapid decline in genetic diversity, a key component of species and ecosystem health in the natural crop populations. These factors negatively affected the species' performance against pathogens, viruses in particular, and caused the widespread cultivation of susceptible species, not only to the known infecting viruses, but also to the emerging viral diseases [25].

The tomato viruses mentioned above have various transmission pathways from infected to healthy plants. But transmission by insect vectors is important for many of them, including some of the most widespread and harmful ones occurring in the Mediterranean region [26–28]. Whiteflies (Hemiptera Aleyrodoidea) include the most important insect vectors of phytopathogenic viruses on tomato crops, and species of the *Bemisia tabaci* (Gennadius) group have a key position due to their responsibility in transmitting, on a variety of crops, in a persistent and circulative manner, over 300 plant viruses primarily belonging to the genus *Begomovirus* within the family *Geminiviridae* [29–31].

Commonly known as the sweet potato whitefly, what is still called *B. tabaci* is, in fact, a cryptic species complex comprising at least 40 morphologically indistinguishable species, which differ from each other in many important biological traits, such as their host plants preference, reproductive capacity, and also virus transmission efficiency [32–36]. This vector is widely distributed in tropical and subtropical regions and in the Mediterranean basin [37–39]. Recent studies carried out in Sicily showed that two mitochondrial variants (Q1 and Q2) of *B. tabaci* MED and the MEAM1 species are the only *B. tabaci* species occurring in agricultural areas of this region, with MED Q1 having been found almost across the island, MED Q2 nearly exclusively detected in the Ragusa province (southeast Sicily), and MEAM1 being apparently almost rare and occurring only in the western part of the island [40].

To date, the current phytovirus control methods, including the genetic improvement approaches, are time-consuming and still vague owing to viral infection complexity, their speedy evolution and pathogenesis peculiarities [41]. This complexity is fueled by the combination of climate change, rising global trade and more interconnected agricultural sectors leading to the emergence of devastating emergent viruses [42]. For viruses which are transmitted by insects, control of the vectors is essential and involves an integrated approach (IPM), especially combining chemical, biological, and cultural/physical methods. Chemical insecticides are widely used due to the high transmission efficiency of the insect vectors involved and the consequent low tolerance threshold they have on susceptible crops; but they often lead to resistance issues, which make chemical control

problematic [43]. Classical biological control using natural enemies, such as parasitoids and predators, offers a sustainable alternative [44], although rarely does such a method allow dramatic reductions in the population of insect vectors. Cultural practices such as the use of resistant varieties are effective in reducing whitefly populations and the consequent virus incidence [45], as are physical methods, including soil mulches or photo-selective covers for greenhouses [46].

However, prevention through early detection and containment remains one of the most effective control strategies against infectious diseases outbreaks [41]. Smart technologies, that connect both scientists and farmers, will improve phytosanitary conditions and significantly contribute to safeguarding tomato production and consequently ensure food security.

Based on the above considerations, it is essential to enable early diagnosis, spatially distributed surveillance, and timely phytosanitary interventions, in order to strengthen sustainable and resilient tomato crop protection. In this context, several studies have been conducted to develop early molecular diagnostic methods for detecting major viral diseases in vegetable crops, such as tomato [9,10,14]. These methods have been used for detection and monitoring in Sicily. Moreover, several different approaches have been already developed in recent years as new technologies for plant pathology, such as sensors platforms for on-field monitoring, volatile organic compound (VOC) analysis, microfluidic-based devices, wearable sensors, IoT and remote sensing technologies [47]. However, to date, they have not been integrated into a decentralized diagnostic and monitoring system that can also be used without skilled personnel supervision, equipped laboratories, and reducing the overall cost per analysis.

Hence, the main objective of the current work was to establish an efficient, sensitive, inexpensive, and easy-to-use decentralized diagnostic network in Sicily, to monitor the most common tomato viral diseases both on the plants and the potential insect vectors.

To that end, an initiative for a multi-Sicilian province network system was started by the central monitoring academic laboratory (CMA-Lab, "Bruno Rosciglione" Plant virology laboratory at SAAF Department, University of Palermo) to manage and monitor different points of care (PoCs) located in the Agrigento, Ragusa, Siracusa and Trapani provinces, with the aim of screening for the most commonly infecting tomato viruses in Sicily.

2. Materials and Methods

2.1. Setting up a Multi-Province Network for Real-Time Detection of Major Tomato-Infecting Viruses

2.1.1. Setting up Points of Care and Diagnostic Networks

In order to monitor in real time the incidence and distribution of the major viral diseases in tomato cultivations, a system was established for collecting, tracking, analyzing, and reporting data from multiple Sicilian tomato producers across four provinces (Agrigento, Ragusa, Siracusa and Trapani). One fixed point-of-care (PoC) screening site was set up in each province, resulting in a total of four installed PoCs. All PoCs were subsequently connected to the CMA-Lab by a cloud-based system.

Each PoC consisted of an air-conditioned space equipped with windows for ventilation and a workbench for conducting analyses, provided by a local producer's organization in each province, equipped with the necessary instrumentation, disposables, and reagents for molecular analysis (Table 1).

In detail, a DC-powered, portable, miniaturized smart device HYRIS bCUBE® (Hyris Ltd., London, UK), was installed in each site. The device enabled instant pathogen tracking using real-time loop-mediated isothermal amplification (LAMP/RT-LAMP) and/or real-

time polymerase chain reaction (real-time PCR/RT-PCR) methods. Molecular analysis results were transmitted back to the CMA-Lab. The process relied on wireless coupling of the smart device to CMA-Lab through its integrated cloud platform, the Hyris bDATA™ service version 2.0, which allowed the Hyris System™ to store and aggregate data, and providing easy access to analysis results and insights.

Table 1. Installed equipment, disposables and tools for real-time tomato virus monitoring network.

Equipment	Description	Location
bCUBE® devices (Hyris Ltd., London, UK) supplied with 12 Volt rechargeable battery	Miniaturized thermal cycler for virus tracking, capable of performing LAMP/RT-LAMP and real-time PCR/RT-PCR analysis	PoCs in Agrigento, Ragusa, Siracusa, and Trapani provinces
Hyris bDATA™ service v2.0	Cloud-based platform for storing and analyzing data collected from bCUBE® devices	CMA-Lab
bAPP web software v2.0	Web application for controlling and managing bCUBE® devices remotely	CMA-Lab
Portable devices	Devices for remotely monitoring and controlling bCUBE® devices	Operators' portable devices (e.g., smartphones, tablets)
Ready-to-use kits	Pre-assembled kits for sample collection, preparation and analysis, designed by CMA-LAB to work with bCUBE® devices	PoCs
Hand homogenizer	Homogenizer hand model with ceramic balls for sample preparation	PoCs
Single hole puncher	Tool for leaves sampling	Operators' portable tool
100–1000 µL micropipette	Variable volume micropipette for reagent dispensing	PoCs
10–100 µL micropipette	Variable volume micropipette for reagent dispensing	PoCs
0.5–10 µL micropipette	Variable volume micropipette for reagent dispensing	PoCs
100–1000 µL micropipette tips	Micropipette tips for reagent dispensing	PoCs
10–100 µL micropipette tips	Micropipette tips for reagent dispensing	PoCs
0.5–10 µL micropipette tips	Micropipette tips for reagent dispensing	PoCs

The bCUBE® is a miniaturized and portable device that can be monitored and controlled remotely from any device with an Internet connection. Using a 36-well cartridge, up to 34 samples (plus positive and negative controls) can be analyzed simultaneously in a 10–25 µL final reaction volume in less than 60 min for real-time LAMP and 120 min for real-time PCR protocols, via two fluorescence detection channels (FAM/HEX). For each PoC, the Internet connection was provided by a 4G portable router, capable of ensuring sufficient data transmission bandwidth for transferring results to the cloud system. To prevent any loss of data during analysis due to a temporary lack of Internet connection, the bCUBE® system is equipped with internal memory (32 GB) for local data storage. Data are immediately transferred to the cloud system once the connection is reestablished.

CMA-Lab was identified as an 'Administrator', having full permissions to remotely operate and control, via the online bAPP software version 2.0 interface, all of the connected groups and users operating the distributed bCUBE® devices. The bAPP is a web-based application that can be accessed across multiple platforms (Windows, Linux, MacOS, iOS, and Android) in order to create, manage custom recipes (including all of the protocols set up by the CMA-Lab) and/or launch analyses on the available devices according to CMA-Lab permissions. Each operator interacted only with their assigned bCUBE, which was directly allocated by the CMA-Lab to a shared work group container, named 'Swarm', that collects analyses and stores data. The pretrained operators selected the desired diagnostic

protocol for the available operating bCUBE and entered the loaded wells' information, such as sample name, type and well color. Once the thermal protocol, previously established by the CMA-Lab, was executed by the operators, the analysis ran directly alongside the real-time status of the device.

A detailed live analysis view page was shown, in which results were automatically visualized and stored in real time in specific tabs. Each step of the analysis was accessed individually, such as the fluorescence measurements, which were updated in real time as the analysis progressed. The virus detection was achieved through optical measurement of the sample's fluorescence, emitted by specific fluorophores present in the reagent mixtures. Once the analysis was completed, results were displayed as labels for each sample, and a detailed PDF report file was generated.

Since the key to successful analysis elaboration relies on the correct use of the device, regardless of the fact that it is easy-to-handle even by unskilled personnel, a quick in situ formation was provided to each operator of the aforementioned provinces, following a unidirectional sample processing/analysis workflow in order to reduce the potential risk for contamination.

2.1.2. Setting up of 'Ready-to-Use' Kits

Four major tomato-infecting viruses were selected for monitoring tomato plants during this study, due to their high diffusion in Sicily: TSWV, ToLCNDV, ToBRFV and STV. For analyses on insect vectors, only ToLCNDV was considered.

CMA-Lab developed and supplied all selected PoCs with specific ready-to-use real-time RT-PCR and RT-LAMP kits for virus detection. The final reaction volume for real-time RT-PCR was 20 µL and the mixture was prepared as follows: three µL of sample, 10 µM of each primer, 0.25 mM of TaqMan probe, 5 µL of 4X CAPITAL™ 1-Step qPCR Probe Master Mix (Biotechrabbit GmbH, Berlin, Germany), 1 µL of 20X RTase with RNase Inhibitor and nuclease-free water to reach final volume. For the real-time LAMP assay, the reaction mixture was prepared as follows: 0.2 µM each of F3 and B3 primers, 1.6 µM each of FIP and BIP, 0.4 µM each of LF and LB, 15 µL of LAMP Isothermal Master Mix (Optigene Limited, West Sussex, UK), 3 µL of sample, and nuclease-free water to reach the final volume.

Each kit allows for the analysis of up to 36 reactions and includes the following components: real-time RT-PCR or RT-LAMP/LAMP master mix with specific primers and probes (Table 2), one positively charged nylon membrane (10 × 8 cm Hybond®-N+ hybridization membrane, GE Healthcare, Chicago, IL, USA), two 50 mL tubes containing general extraction buffer—GEB (1.3 g L^{-1} sodium sulfite anhydrous, 20 g L^{-1} polyvinylpyrrolidone MW 24–40.000, 2 g L^{-1} albumin chicken egg grade II and 20 g L^{-1} Tween 20; pH 7.4), 34 extraction bags for sample homogenization, 10 mL of glycine buffer (GB) (0.1 M glycine, 0.05 M NaCl and 1 mM EDTA) for sample preparation, virus synthetic positive (PC) and negative (NC) controls, and one multi-well cartridge supplied with the aluminum foil to seal it, provided jointly with a detailed protocol sheet.

All kits were pre-tested and validated at CMA-Lab, using virus synthetic positive (PCs) and negative controls (NCs), before being distributed at the different PoCs, in order to assess their reliability [8,10,14,48] and absence of any contamination. For each produced kit, PCs and NCs were analyzed in duplicate in three independent assays.

Table 2. Primer set and probe sequences included in ready-to-use kits for detection of screened viruses by real-time RT-LAMP/LAMP and real-time RT-PCR.

Virus	Detection Method	Primer Name	Sequence (5′-3′)	Genomic Position (nt)	Target Gene	Amplicon Size (bp)	References
TSWV	Real-time RT-LAMP	TSWV-F3	TTCAGCACAGTGCAAACT	2218–2235	Coat protein	220	[14]
		TSWV-B3	CTTTGATTCAAGCCTATGGATT	2416–2437			
		TSWV-FIP	GCAATAAGAGGTAAGCTACCTCCCCCTCTCGATGATGCAAAGT	2252–2266 2306–2330		–	
		TSWV-BIP	ATGATCAGTGTTGTCTTGGCTATATTTCCTTGGTGTCATACTTCT	2350–2373 2396–2414			
		TSWV-LF	AGCATTATGGCAAGTCTCACAG	2374–2395		–	
		TSWV-LB	ATCAGGATGCAAAATACAAGGACC	2282–2305			
ToLCNDV	Real-time LAMP	ToLCNDV-F3	GTGGCATGCTACTGTGAC	821–838	AV1	218	[8]
		ToLCNDV-B3	CCGAATCATAAAAATAGATCCGG	1016–1038			
		ToLCNDV-BIP	CGGCAAGTATGAGAATCATACTGAACAAAGTAGCATACACAGGATT	926–971		–	
		ToLCNDV-FIP	GCCTCTTGTTGATTGTAAACAACATGAGGAACGTATGCATCAAGG	881–925			
		ToLCNDV-LF	CAAACTTCCTAACTAATGCTTGCTC	861–885		–	
		ToLCNDV-LB	TGTTGTATATGGCCTGTACTCATG	961–984			
ToBRFV	Real-time RT-PCR	ToB5520F	GTAAGGCTTGCAAAATTTCGTTCG	5520–5544	Movement protein	101	[10]
		ToB5598R	CTTTGGTTTTTGTCTGGTTTCGG	5598–5621			
		ToB-probe	FAM-GTTTAGTAGTAAAAGTGAGAAT-MGB	5558–5580			
STV	Real-time RT-PCR	STV-F	TGCCTCCCCAGCTGTCA	1191–1207	Coat protein	68	[48]
		STV-R	TGCGTTGGGATAGAGGAGTGA	1238–1258			
		STV probe	6FAM-CGCAACAGAGGTAGAGGCAGAGGCC-TAMRA	1210–1234			

2.1.3. Point-of-Care Analyses

During the period of September 2023–May 2024, five-hundred tomato samples were collected in duplicate from a total of ten producers (50 samples/producer) for each of the surveyed Sicilian provinces (Agrigento, Ragusa, Siracusa and Trapani provinces) and sent to each screening point (PoC) and the CMA-Lab, respectively.

The producers included in the diagnostic network participated in the sampling on a voluntary basis, agreeing to follow our sampling procedures and submit the collected samples to their respective PoC. Among the proposed producers, ten were selected for each province, based on the following characteristics: cultivated area (approximately 10 hectares); tomato crop in greenhouse conditions; and random geographic location within the tomato-growing area of each province. Furthermore, the total area considered in the four provinces represents approximately 10% of Sicilian tomato cultivation in greenhouse conditions.

Each sample was analyzed for the viruses listed in Table 2, for a total of 2000 analyses per province and 8000 analyses overall.

The primary objective was to enable a quick survey by collecting and processing samples through PoC implementation in each province. Therefore, this step aimed to extract, as much as possible, the targeted pathogen's genomic material (DNA/RNA) and, at the same time, minimize contaminants and inhibitory substances that could interfere with the amplification reaction. This was achieved through the use of a novel, quick, and easy sample preparation procedure called 'membrane spot crude extract' [49], adapted to tomato samples for an equipment-free extraction and deployed directly by the pre-trained operators, using the previously described 'ready-to-use' kits, to avoid the total RNA/DNA extraction using commercial kits. With this extraction method, it is possible to balance technological simplicity with efficient genomic material recovery, reducing the risk of cross-contamination between samples. Specifically, for each sample, a total of ten fresh leaf disks of about 0.5 cm^2 in diameter were sampled from 10 tomato plants (1 leaf disk per plant) with a hole puncher and subsequently homogenized using the HOMEX 6 hand homogenizer (Bioreba, Reinach, Switzerland), with 3 mL of GEB inside the extraction bag. An aliquot of 5 µL of the homogenate was spotted directly onto the 0.5 cm^2 Hybond®-N+ hybridization membrane placed inside the 2 mL provided tube, and then allowed to dry at room temperature for 5 min. Afterwards, 250 µL of GB was added to the hybridized membrane and mixed by vortexing for 15 s, then samples were heated at 95 °C for 10 min. After incubation, three µL of the obtained solution was mixed with the provided master mix and used for the real-time LAMP and/or real-time RT-PCR reactions. Positive and negative controls were included in each test. The reaction mixture was loaded inside the cartridge reaction wells and carefully sealed on the top with the black aluminum adhesive foil; the analyses were launched by the web software bAPP on cloud and results were visualized in real time.

To further prevent contamination and ensure proper kit usage, operators followed a set of best practices, including: storing the kit components at room temperature in a dry place and the master mix at −20 °C until use; handling the nylon hybridization membrane with tweezers and latex gloves, avoiding direct contact with bare hands; whenever possible, the sample extraction and analysis steps should be performed by two different operators, otherwise, operators were instructed to change gloves before distributing the reaction mixture; conducting sample preparation in a different location/room from where the reaction mixture is distributed in the 36-well cartridge; ensuring complete adhesion of the aluminum adhesive black seal to the metallic surface of the cartridge; starting the analysis run within ten (10) min of cartridge loading; and never removing the adhesive foil cover

from the cartridge after use, or reusing cartridges or reagents from previous runs, in order to avoid the risk of contamination.

Briefly, both TSWV and ToLCNDV were screened by real-time RT-LAMP and real-time LAMP, respectively, and reactions were performed following the kit's instructions. The assay was conducted at 65 °C for 60 min, and fluorescence was acquired every 60 s. ToBRFV and STV were monitored employing real-time RT-PCR analysis. For ToBRFV, the cycling conditions included reverse transcription at 45 °C for 10 min, initial denaturation at 95 °C for 10 min, 45 cycles of denaturation at 95 °C for 5 s and annealing at 60 °C for 60 s with fluorescence measured at the end of each cycle [10]. For STV, the thermal cycling conditions consisted of reverse transcription at 42 °C for 15 min, incubation at 94 °C for 10 s and 40 cycles of 94 °C for 5 s and 60 °C for 20 s [48]. Tomato samples were considered positive to TSWV and ToLCNDV when the amplification curve reached the plateau within 60 and 45 min, respectively, and for ToBRFV and STV, samples were considered positive when the cycle threshold (Ct) value was <35 and <40, respectively.

In addition, the presence of ToLCNDV in a whitefly vector of the species group *B. tabaci* has been assessed in ten tomato crops grown under greenhouse in the sampling area of Ragusa province. To this aim, two hundred adults of the whitefly were collected from each of the ten selected tomato crops (20 adults/plant, on 10 plants/crop) using a mouth aspirator (John W. Hock Company, Gainesville, FL, USA) and quickly transferred into 1.5 mL Eppendorf tubes. Subsequently, two groups of ten insects per crop were separated to carry out, per each crop sample, parallel analyses in PoC and laboratory (CMA-Lab) conditions. In PoC conditions, the ten insects from each sample were squeezed directly onto the 0.5 cm^2 Hybond®-N+ hybridization membrane, and then analyzed for ToLCNDV, as described above; in laboratory conditions, each sample was stored at −20 °C and sent to the CMA-Lab (approximately five–six hours after whitefly collection), where DNA extraction and molecular analyses were conducted as described below.

2.2. Species Identification of the Whitefly Vector

Although the exclusive presence of *B. tabaci* MED has recently been reported in the investigated area in the Ragusa province [40], a preliminary check was performed on the whitefly species involved in these studies through molecular characterization of the population occurring in a randomly selected crop among the ten investigated (locality Marina di Acate: 36°58′54.2″ N, 14°23′44.0″ E). Here, fifty unsexed adult whiteflies were collected from tomato plants using a mouth aspirator (John W. Hock Company, Gainesville, FL, USA) and quickly transferred to the CMA-Lab, where the total DNA was extracted from single adults following the method described by Walsh and co-workers [50] and De Barro and Driver [51]. The mitochondrial cytochrome oxidase I (mtCOI) gene (about 710 bp) was amplified using universal primers LCO1490 and HCO2198 [52]. For each sample, the 10 µL reaction volume contained 5 µL of FailSafe™ 2X PreMixes buffers (Lucigen, Middleton, WI, USA), 3.75 µL of DNA, 0.25 µL of Taq polymerase, and 0.5 µL of each forward and reverse primer. The PCR was performed with the denaturation at 96 °C for 5 min, followed by 35 cycles, each consisting of denaturation for 45 s at 96 °C and annealing for 60 s at 45 °C, with final extension for one minute at 72 °C, followed by final extension for 10 min, at 72 °C. PCR-amplified products (10 µL) were visualized with 0.9% agar-gel electrophoresis (5 µL), and those with the target fragment were selected for sequencing. Successfully amplified DNA (5 µL) was purified and sequenced by BMR Genomics (Padova, Italy). Unique sequences were identified, and a BLAST search was performed on the NCBI website (https://www.ncbi.nlm.nih.gov, accessed on 5 September 2024) using default settings. Finally, the *B. tabaci* identity was determined by alignment to the mtCOI reference dataset [53].

2.3. Data Collection and Processing

All samples were analyzed using the bCUBE® devices, and the data generated by the bCUBEs from the PoCs were remotely retrieved from the Hyris bAPP web application by the CMA-Lab, to enable de-centralized analyses and results evaluation of each conducted assay.

In detail, all reports related to the conducted analyses were collected through the Hyris bAPP interface. Geographical information obtained via Keyhole Markup Language (KML)-based information resources were retrieved from the GADM website (https://gadm.org/index.html) (accessed on 10 January 2025) and subsequently plotted using Google Earth software [54], to display the incidence and distribution of the four selected tomato viruses in each province. The incidence of ToLCNDV on all *B.* sp.gr. *tabaci* adults collected in the Ragusa province was also evaluated and validated.

In order to analyze the data, Python programming language 3.12.13 alongside key libraries including NumPy 2.0.2, Pandas 2.2.2, Matplotlib 3.10.0 and SciPy 1.16.3 was employed. NumPy was utilized for efficient numerical computations, while Pandas facilitated data manipulation and organization. The chi-square statistic was computed using SciPy to assess the association between categorical variables. Matplotlib was used to produce a figure about the effect size.

2.4. Analysis Validation

Furthermore, to assess the analyses' specificity, sensitivity, and fitness-for-purpose under PoC conditions and validate the results obtained employing the ready-to-use kits and bCube portable thermal cyclers under resource-poor settings, tomato samples and collected adults of *B.* sp.gr. *tabaci* were subsequently analyzed at the CMA-Lab.

To enable a structured comparison between laboratory-based and PoC-based diagnostic systems for tomato viral disease detection, a set of key performance indicators (KPIs) was defined based on established principles for diagnostic test evaluation. These included analytical performance parameters: sensitivity, specificity, accuracy and agreement with the reference method, as well as operational parameters relevant to decentralized agricultural deployment, namely time to result, cost per test, portability, skill requirements and robustness. Performance values for each KPI were deduced from previously published validation studies of the molecular assays considered; when multiple sources were available, representative or average values were used. A semi-quantitative scoring system ranging from 1 (poor) to 5 (excellent) was applied using predefined threshold intervals summarized in Supplementary Table S1 and derived from performance ranges reported in the diagnostic literature, following established principles for diagnostic accuracy evaluation and reporting [55,56] and their application in molecular plant disease diagnostics and field-deployable systems [57,58]. Higher scores indicate better performance. This framework provides a comparative evaluation of laboratory and PoC-based diagnostic systems under agricultural deployment conditions.

In laboratory conditions, the nucleic acid extraction started from ≈100 mg of fresh tomato leaf tissue, homogenized in a sample extraction bag using the HOMEX 6 homogenizer (Bioreba, Reinach, Switzerland), with 1 mL extraction buffer (1.3 g sodium sulphite anhydrous, 20 g polyvinylpyrrolidone MW24–40.000, 2 g chicken egg chicken albumin grade II, 20 g Tween-20 in one L of distilled water; pH 7.4). In the case of *B. tabaci*, 10 adults per sample were used for homogenization. Four hundred µL of the sample extract were added to the same lysis buffer volume provided in the kit, and the manufacturer's protocol was followed from this step. Regarding total DNA extraction, the GenUP® Plant DNA kit (Biotechrabbit GmbH, Berlin, Germany) was used following the manufacturer's instructions, with minor modifications, while total RNA was extracted using the NucleoSpin® RNA Plant kit (Macherey-Nagel GmbH & Co., Dueren, Germany), following the man-

ufacturer's instructions. Lastly, each sample was resuspended in 100 µL nuclease-free water. Total DNA/RNA concentration was quantified twice using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and the final concentrations were adjusted to ≈50 ng/µL. The molecular assays were performed in a Rotor-Gene Q 2plex HRM Platform Thermal Cycler (Qiagen, Hilden, Germany), using 3 µL of total DNA/RNA (≈50 ng/µL) previously purified, and nuclease-free water to reach the final volume. Final reaction volume and mixture were prepared as previously described.

Results obtained at the CMA-Lab following the molecular protocols described in Table 2 were then compared with the analyses performed at the different PoCs using the ready-to-use kits.

3. Results

3.1. Setting up a Multi-Province Network for Real-Time Detection of Major Tomato-Infecting Viruses

3.1.1. Setting up of Points of Care and Diagnostic Network

As a result of the initiative to establish a multi-province network for real-time tomato virus monitoring, equipment, disposables and tools reported in Table 1 were installed at the selected PoC locations.

A Wi-Fi connection was paired to each portable thermal cycler and the portable device (smartphone/tablet) in order to ensure a stable Internet connection and enable real-time data exchange. Subsequently, the implemented PoCs were integrated into their respective "swarm" working groups, which were remotely managed by the CMA-Lab. Using the bAPP Web Software, the CMA-Lab monitored and validated, in real time, the analysis results.

Afterwards, the training of the operators was carried out at each PoC. As illustrated in Figure 1, the training covered the main operational features of the portable thermal cycler, the use of the sample loading cartridges, the web app for managing analysis, the components of the ready-to-use kits, the use of the micropipettes/consumables needed for sample preparation, as well as the subsequent steps for analysis setup and results visualization.

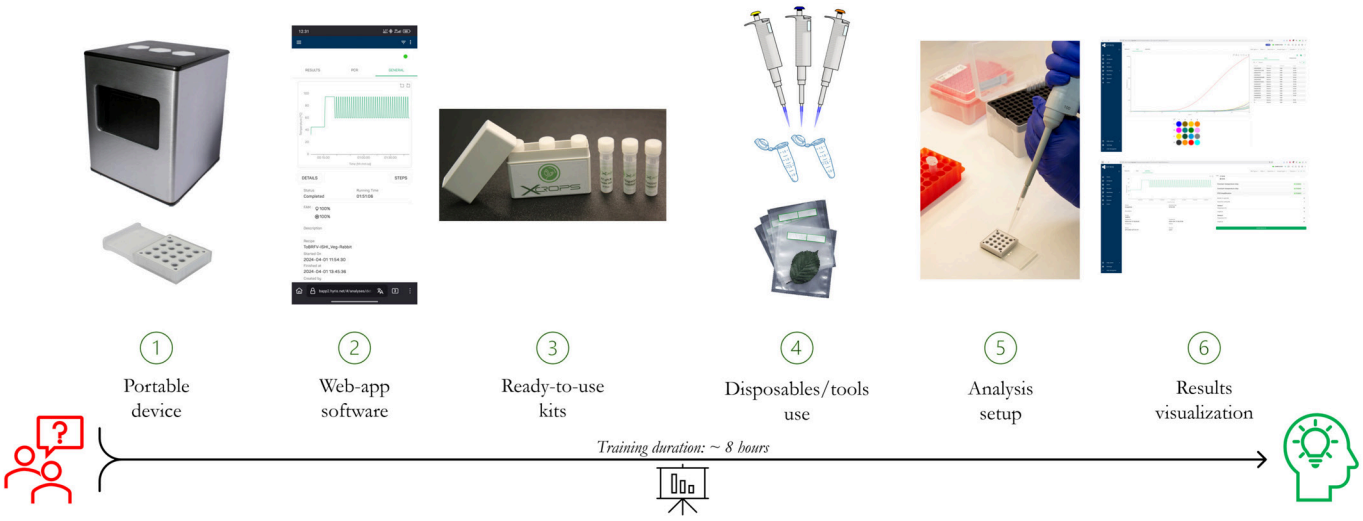

Figure 1. Training workflow for PoC personnel.

Although the amount of time required for training can vary depending on the procedure and the molecular technique, the training at each PoC had an average duration of eight hours, during which the operators became proficient in all of the necessary steps of the analytical process, demonstrating that they could successfully perform the assays, through analyses of positive and negative control samples, before being capable to analyze biological samples without supervision.

3.1.2. Setting up of 'Ready-to-Use' Kits

Fifteen kits for each virus were produced and tested by the CMA-Lab operators, and subsequently supplied to each PoC, for a total of 60 kits/PoC. CMA-Lab validation confirmed the reliability of the ready-to-use kits and the absence of any contamination that could compromise the results.

Each kit enables the rapid on-site analysis of up to 34 samples (Figure 2), allowing farmers to quickly detect viral pathogens (Table 3) and make timely management decisions to minimize production losses.

Figure 2. Ready-to-use molecular diagnostic kit.

Table 3. Test duration of molecular diagnostic tests used in this work.

Virus	Detection Method	Sample-to-Result Test Duration (min)
TSWV	Real-time RT-LAMP	60
ToLCNDV	Real-time LAMP	30
ToBRFV	Real-time RT-PCR	120
STV	Real-time RT-PCR	120

3.1.3. Point-of-Care Analyses

After the producers submitted the collected samples to their respective PoC, all pre-trained PoC operators were able to extract the samples, following the recommendations reported in Section 2.1.3, and initiate analyses within approximately 10–15 min, obtaining results in 60–120 min, depending on the reaction protocol used (Figure 3).

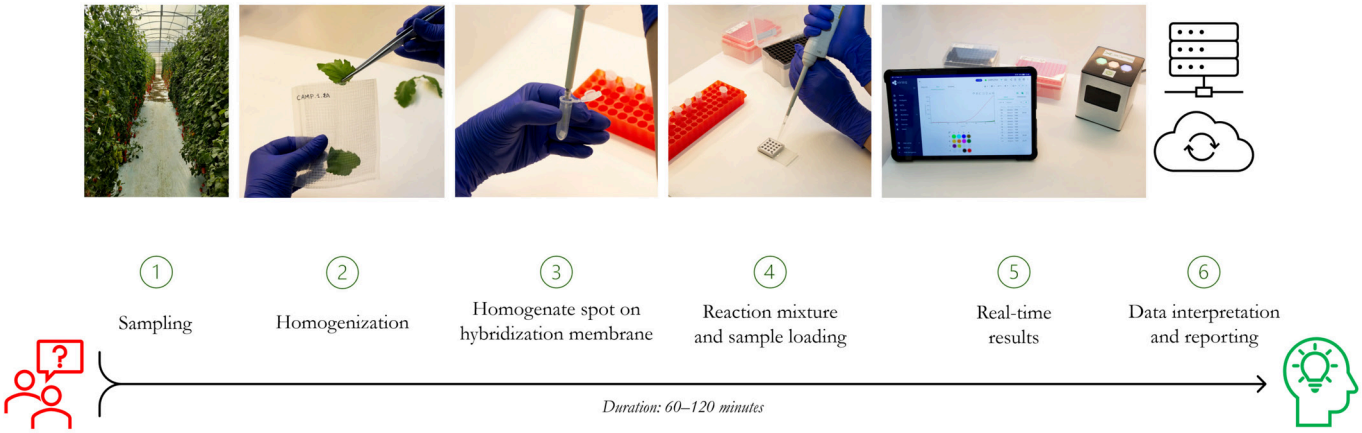

Figure 3. Analysis workflow for portable nucleic acid amplification.

3.2. Species Identification of the Whitefly Vector

The DNA was extracted from 50 whitefly individuals, out of which 43 produced a sequence of sufficient quality to perform molecular analysis and identify the species as *B. tabaci* MED. This does not contradict what is claimed by Milenovic and co-workers [40] and Parrella and co-workers [59], who asserted this as the most widespread species living on solanaceous in the Mediterranean area, and particularly in Sicily. Therefore, to *B. tabaci* MED we ascribe the whole whitefly material processed in this study.

3.3. Data Collection and Processing

The results from tomato samples analyzed at the PoCs across the four provinces showed variable virus incidence and distribution depending on the virus (Figure 4).

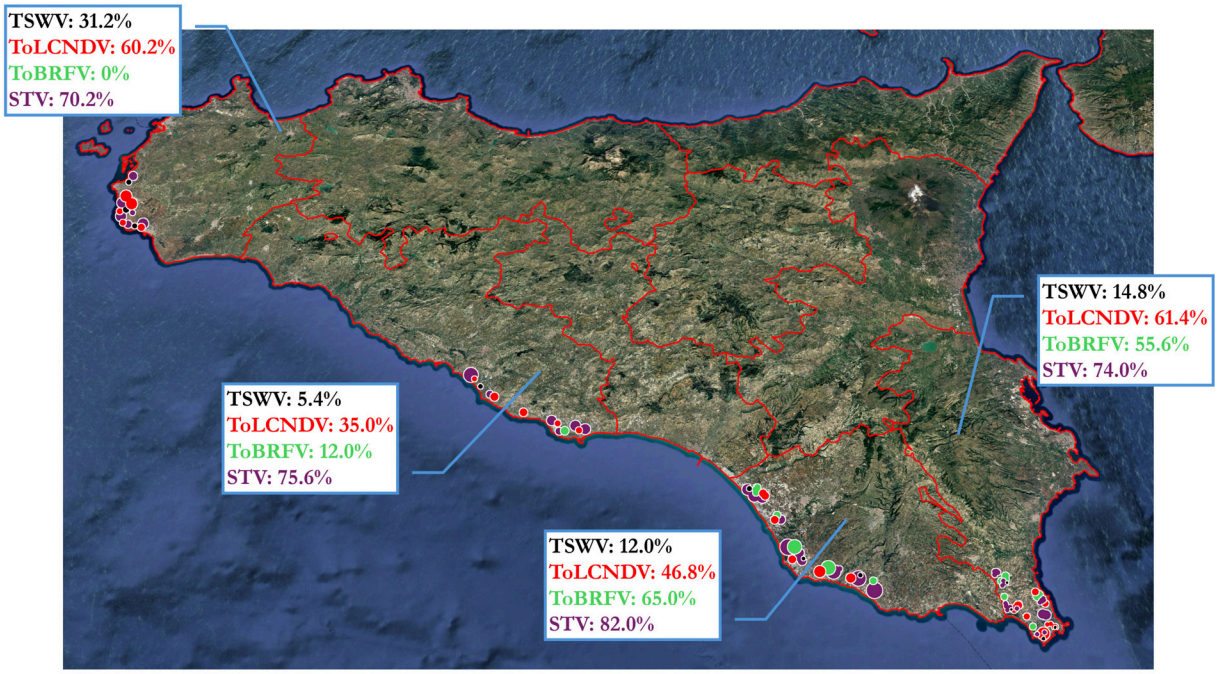

Figure 4. Map of the Sicilian provinces illustrating the distribution and the incidence percentage of the major tomato viruses assessed in this study (black: tomato spotted wilt virus—TSWV; red: tomato leaf curl New Delhi virus—ToLCNDV; green: tomato brown rugose fruit virus—ToBRFV; and purple: southern tomato virus—STV). Basemap source data retrieved from GADM website—https://gadm.org/data.html (accessed on 10 January 2025).

As shown in Table 4, STV infection had the highest incidence, with rates above 70% in all provinces. In detail, the highest rate was registered in Ragusa province (82%) with a total of 410 positive samples, followed by Agrigento (75.6%), Siracusa (74.0%) and Trapani (70.2%).

A different incidence was observed regarding ToBRFV; in Ragusa and Siracusa provinces the highest percentages were observed (65% and 55.6%) with a total of 325 and 278 positive samples, respectively, followed by Agrigento (12%), while Trapani showed no positive cases.

Regarding ToLCNDV infection, the virus was found to be widely distributed across all provinces; the lowest incidence was observed in Agrigento (35.0%) with 175 positive samples, while the highest incidences (60.2% and 61.4%) were observed in Trapani and Siracusa, respectively. Lastly, TSWV had the lowest overall incidence among all provinces, ranging from 5.4% in Agrigento to 31.2% in Trapani.

Table 4. Positive tomato samples and incidence percentage of the major tomato viruses assessed in Trapani, Agrigento, Ragusa and Siracusa provinces.

Virus	Trapani		Agrigento		Ragusa		Siracusa	
	Positive Samples	Incidence (%)	Positive Samples	Incidence (%)	Positive Samples	Incidence (%)	Positive Samples	Incidence (%)
TSWV	156	31.2	27	5.4	60	12.0	74	14.8
ToLCNDV	301	60.2	175	35.0	234	46.8	307	61.4
ToBRFV	0	0	60	12.0	325	65.0	278	55.6
STV	351	70.2	378	75.6	410	82.0	370	74.0

Regarding *B. tabaci* MED in Ragusa province, the presence of ToLCNDV was confirmed in adults collected in nine out of ten tomato crops, with an average incidence of approximately 47.0% (Table 5).

Table 5. Incidence of ToLCNDV in *Bemisia tabaci* MED adults infesting tomato crops in Ragusa province.

Tomato Crop ID	*B. tabaci* MED Positive Samples	Incidence (%)
RAG-1	4/10	40
RAG-2	3/10	30
RAG-3	8/10	80
RAG-4	10/10	100
RAG-5	4/10	40
RAG-6	8/10	80
RAG-7	0/10	0
RAG-8	2/10	20
RAG-9	3/10	30
RAG-10	5/10	50

The between-province analysis using the chi-square test carried out for each considered virus highlights that the observed incidences are statistically significant (p-values < 0.01). In Figure 5, the computed p-values are reported, shown at the top of each corresponding bar, along with provide the strength of the difference by a power analysis, in particular, the effect size by Cramer's V.

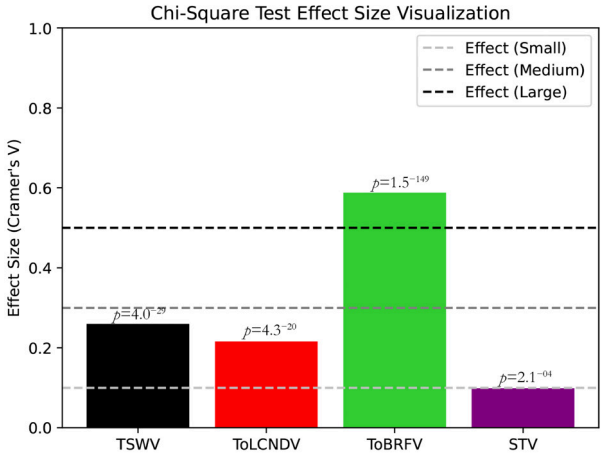

Figure 5. Bar chart illustrating the effect sizes (measured by Cramer's V) for the four different viruses, computed by carrying out a between-province chi-square test. At the top of each bar, the related p-values are shown.

It can be observed that this strength ranges from small for STV to moderate for TSWV and ToLCNDV and large for ToBRFV.

3.4. Analysis Validation

Finally, the KPI evaluation demonstrated comparable results between laboratory and PoC conditions for most analytical and operational parameters. In detail, sensitivity, specificity, accuracy, robustness and agreement with the reference method consistently revealed high performance in both settings, with scores aligned with the predefined evaluation framework. In contrast, operational parameters such as time to result, cost per test, portability, and skill requirements were significantly higher under PoC conditions, with a maximum efficiency score of five. These differences are mainly attributable to reduced infrastructural requirements and simplified workflows in PoC-based systems compared to laboratory settings (Table 6). A slight reduction in analytical sensitivity was observed in PoC conditions due to the use of rapid sample preparation methods. Nevertheless, the reliability of results remained uncompromised. Across all performed assays, a minor increase in Ct values (+2 amplification cycles) for the real-time PCR and a delay in the reaction plateau (+2/3 min) for LAMP assays were observed when compared to laboratory analysis, using nucleic acids (DNAs and RNAs) extracted with commercial kits.

Table 6. Evaluation and comparison of selection parameters between laboratory and point of care (PoC) conditions.

Key Performance Indicators (KPIs)	Laboratory Conditions	Point-of-Care Conditions
Sensitivity	5 (Excellent sensitivity)	4 (High sensitivity)
Specificity	5 (Excellent specificity)	5 (Excellent specificity)
Accuracy	5 (Excellent accuracy)	5 (Excellent accuracy)
Robustness	5 (Stable under controlled conditions)	5 (Stable under field conditions)
Time to result (min)	150–200 (Score 2)	<120 (Score 5)
Agreement with reference method (%)	>98 (Score 5)	>98 (Score 5)
Cost per test (€)	25–35 (Score 2)	<10 (Score 5)
Portability	1 (Non-portable laboratory setup)	5 (Fully portable system)
Skill requirements	1 (Expert operator required)	4 (Basic training sufficient)

Overall, PoC assays required only basic operator training, supporting their applicability for decentralized surveillance of tomato viral diseases under resource-limited agricultural conditions.

4. Discussion

The development of a multi-province diagnostic network using bCUBE® devices and cloud-based services for real-time virus monitoring in tomato crops represents a further step forward in plant pathology diagnostics. Compared to the more rigid centralized laboratory diagnostics that face geographical and temporal barriers [47], this system is more flexible and agile. The integration of Wi-Fi-enabled portable thermal cyclers and cloud data platforms like Hyris bDATA™, provided remote data validation, which is a critical aspect missing from many existing studies. Furthermore, the implemented operator training process, which lasts for 8 h, is similar to Yadav and Yadav [60], who highlighted the need to train users to improve their accuracy and efficiency. However, in this study, the designed system allowed CMA-Lab to perform remote monitoring and validation, enabling real-time data assessment alongside prompt and effective intervention.

The developed ready-to-use kits for viral detection demonstrated the capability to diagnose up to 34 samples simultaneously with various molecular diagnostic techniques (real-time LAMP/PCR) integrated into a single kit, which provides greater flexibility for detecting various viruses such as TSWV, ToLCNDV, ToBRFV and STV. This innovative approach was demonstrated to be unlike prior work from Buja and co-workers [47], which was based on single-pathogen detection and did not include ready-to-use kits. The current approach facilitated the on-site sample processing with minimal preparation time, a significant improvement if compared to traditional methods which often require complex manual reagent preparation and analysis [61].

In this study, the use of the membrane spot crude extract method demonstrated an exemplary equilibrium between ease of operation and effective recovery of genomic material across the various PoCs. This is crucial especially in plant virology PoC diagnostics, where low sensitivity because of the crude extract and high complexity for field application due to the user-centric protocols are huge challenges. Unlike previous studies where sample preparation needed specific instrumentation and complex extraction buffers [62], our method streamlined the removal of inhibitory components without compromising diagnostic reliability or accuracy. The operational protocol provided, particularly the strict contamination prevention measures, defined the handling procedures, which constitutes a practical achievement in PoC workflow. Notably, the sample-to-result turnaround time (5–10 min for extraction and 60–120 min for results) is competitive or even faster than other portable molecular assays, where LAMP assays in the field took up to 2.5 h. This highlights the method's suitability towards immediate decision-making in real-time crop management, optimally in remote or resource-constrained agricultural environments. A key strength of the adopted approach in this study is the reliability (confirmed by parallel analyses conducted by CMA-Lab) and simplicity of use, even with minimal personnel training. By enforcing a standardized set of good laboratory practices appropriate to the PoC conditions, the study ensured high performance and reduced risks of cross-contamination, which is a common limitation for decentralized diagnostic methods [63]. In addition, the ability to carry out reactions in a small 36-well cartridge system increases throughput while still being portable, filling a scalability gap that many earlier PoC platforms lacked.

Furthermore, the broad field survey of the key tomato viruses across four Sicilian provinces revealed that STV is the most widespread virus, with a high incidence (>70%) across all provinces, confirming findings in previous studies, and evidence that STV typically is seed-borne, which leads to latent infections and makes it very difficult to eradicate [22]. The high incidence of STV in Ragusa province (82%) makes a case for the propagation of healthy seeds and suggests possible seed-borne introduction or an inability to eradicate STV from greenhouse systems. The variable incidence of ToBRFV aligns with its known very quick emergence and spread in some areas. The high incidence observed in Ragusa (65%) and Siracusa (55.6%) provinces aligns with previous findings in other Mediterranean regions [13], suggesting continuous outbreaks likely related to contaminated propagation material and/or mechanical transmission. However, the complete absence of ToBRFV in Trapani province, despite its proximity to other affected provinces, suggests either effective containment and management practices, limited vector presence or a delayed introduction, which is an important point that will guide targeted phytosanitary interventions. ToLCNDV revealed a widespread incidence in all provinces. In detail, the highest incidences were reported from Siracusa (61.4%) and Trapani (60.2%) provinces. These findings align with previous reports of the geographic ToLCNDV expansion in Southern Europe and highlight the fast adaptability of this begomovirus to many agroecological zones [64]. Notably, in Agrigento province the virus incidence was lower (35.0%) if compared to other surveyed provinces, potentially indicating the presence of the whitefly

vector and/or different host susceptibility. In addition, the low but notable presence of TSWV, with 31.2% of incidence in Trapani and 5.4% in Agrigento provinces, suggests local transmission dynamics likely related to thrips populations, as previously reported in Italian surveys [65]. Although TSWV is less prevalent, its presence confirms its ongoing circulation and suggests the need for integrated vector management.

In pest control strategies, scouting and sampling techniques are key components of the monitoring process [66,67], as prompt and reliable information on pest presence, density and damage level, as well as on related plant diseases, provides a solid base for decision-making in management programs. In particular, prompt field assessment of density and infectivity of virus vector insects is critical to protect crops from virus diseases, as confirmed by studies in which rapid and sensitive detection methods have been used, such as PCR or LAMP tests [68–70].

In the present study, the real-time LAMP technique has been applied to detect ToLCNDV directly from the insect vector, *B. tabaci* MED, using a set of specific loop primers for the amplification reaction [8]. This diagnostic procedure has shown numerous advantages, mainly in terms of applicability and fast analysis execution performed on insects.

In particular, the confirmation of ToLCNDV presence in *B. tabaci* MED adults retrieved in Ragusa province further supports the crucial role of whiteflies as primary vectors in virus spread. The virus was found in adult whiteflies collected in nine out of ten crops, with incidence rates ranging from 0% to 100% in individual populations. The virus incidence variability is associated with the variable distribution of whitefly populations [71] and highlights the efficacy of the PoC system in early detection of vector-borne diseases. The methodology applied in this work, which involves the use of digital systems for remote analysis of data collected from a wide territory and in different PoCs, has the potential to establish a responsive monitoring network, which is useful not only for managing important key pests, but also for preventing the spread of harmful viral diseases, which could pose a significant threat to crops in risk areas.

Moreover, the comparison between laboratory and PoC conditions, based on a semi-quantitative KPI framework (Table 6; Supplementary Table S1) integrating both analytical (sensitivity, specificity, accuracy, and agreement with the reference method) and operational performance parameters (time to result, cost, portability, robustness, and skill requirements), revealed comparable results for the detection and monitoring of major tomato viruses, with specificity, accuracy, and robustness all achieving the highest score (5). This integrated approach allows for a standardized assessment of diagnostic performance under field-deployed agricultural conditions.

These findings mirror what was reported by Caruso and co-workers [8,14], Rizzo and co-workers [72] and Elvira-González and co-workers [73], who developed or implemented and validated RT-LAMP assays for the detection of ToBRFV, ToLCNDV, TSWV and STV in tomato seeds and/or plants, respectively. However, our current study expanded on the previous findings by testing PoC protocols with an extensive sampling effort, involving pretrained operators, four provinces, and over 2000 samples, therefore reporting high validation of decentralized diagnostic approaches. While sensitivity at the PoCs was slightly affected due to the simplified extraction method (score 4), results remained reliable and valid for monitoring purposes. These findings agree with previous observations done by Notomi and co-workers [74], who reported slight efficiency drops when using crude extracts using the molecular LAMP diagnostic technique. The portable device outperformed the analysis conducted in the laboratory in terms of time, cost, and portability, which achieved a maximum score (5), parameters which are critical factors for rapid decision making. Notably, skill requirement scores (4 in PoCs and 1 in laboratory) were significantly improved following brief operator training, confirming the system's practicality for wide adoption

enabling the full process from sample extraction to amplification within 5 to 10 min. In fact, the duration of the entire "sample-to-result" analysis process—which incorporates three key factors (real-time performance, the number of manual steps, and the level of technological integration) and reflects workflow, process automation, and simplicity—has improved significantly under PoC conditions.

5. Conclusions

The current study is the first to develop and implement a decentralized multi-province diagnostic network using portable devices and cloud-based services for real-time monitoring of various tomato viruses, integrated with operator training and remote data validation for viral disease diagnosis. The deployment of the proposed smart decentralized network will greatly represent a fundamental and instantaneous surveillance tool for preventing the spread of the most threatening tomato viruses in several diagnostic points, and subsequently provide continuous, early, and real-time knowledge of disease epidemiology. By minimizing production losses, this system can contribute to more sustainable and environmentally friendly crop management, avoiding incorrect crop operations and wasted resources by focusing interventions only when necessary and with targeted accuracy.

Moreover, this system could support integrated management strategies by reducing diagnostic delays, improving outbreak containment, and optimizing the use of control measures, ultimately contributing to lower environmental impact and enhanced agroecosystem stability in tomato-growing regions.

Further studies may help strengthen the technological context and demonstrate how distributed diagnostic networks can be integrated within the broader context of smart agriculture and remote crop monitoring systems, such as integration of sensor-based crop monitoring, soil moisture prediction, and data-driven decision support systems, addressing RGB/colorimetric approaches for crop water status monitoring, machine-learning-based soil moisture prediction, sensor-based irrigation management systems, and remote sensing frameworks for precision agriculture.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture16101048/s1, Table S1: Definition and scoring criteria of key performance indicators (KPIs) for analytical (A) and operational (B) evaluation of diagnostic performance. Table S2: Analysis results comparison for both LAMP (TSWV and ToLCNDV) and qPCR (ToBRFV and STV) assays between PoCs and laboratory condition (CMA-Lab). Results were expressed as reaction plateau minute for TSWV and ToLCNDV, and Ct value for ToBRFV and STV.

Author Contributions: Conceptualization, S.D., S.P., C.R. and A.G.C.; methodology, S.D., S.P., C.R. and A.G.C.; software, G.L.B. and A.G.C.; validation, S.P. and C.R.; formal analysis, E.Y., A.F., G.I., M.D.D. and G.L.B.; investigation, E.Y., A.G.C., A.F., G.I., M.D.D. and S.P.; resources, S.D.; data curation, A.G.C., C.R., G.L.B. and S.P.; writing—original draft preparation, A.G.C., E.Y. and S.P.; writing—review and editing, E.Y., A.G.C., A.F., G.I., M.D.D., C.R., G.L.B., S.P. and S.D.; visualization, A.G.C., C.R., S.P. and S.D.; supervision, C.R., S.P. and S.D.; funding acquisition, S.D. All authors have read and agreed to the published version of the manuscript.

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