Sains Malaysiana 52(3)(2023): 757-770
http://doi.org/10.17576/jsm-2023-5203-06

Estimation of Biocidal Potential of Desert Phytopowders for the Management of Citrus Canker

(Anggaran Potensi Biosid Fitoserbuk Gurun untuk Pengurusan Kanker Sitrus)

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Received: 2 March 2022/Accepted: 27 February 2023

ABSTRACT

Citrus is one of the most important fruit crops, throughout the world. It is a rich source of antioxidants and vitamin C. Citrus canker is a potential threat to its successful production. In current study, ten desert phytopowders including *Xanthium strumarium*, *Dipterygium galucun, Leptadenia pyrotechnica, Haloxylon recurvum, Suaeda fruticosa, Salsola baryosma, Citrulus colocynthis, Abutilon indicum, Aerva javanica,* and *Calotropis procera* at three different concentrations (5.0, 7.5 and 10.0%) were evaluated under *in vitro* conditions against *Xanthomonas citri* pv. *citri*. Among all phytopowders, *X. strumarium* and *S. fruticosa*, showed maximum inhibition zone (40 mm) followed by *S. baryosma* (38.50 mm) *C. colocynthis* (37 mm), *Abutilon indicum* (34 mm), *H. recurvum* (32 mm), *D. galucun* (30.5 mm), *A. javanica,* (29.50 mm), *L. pyrotechnica,* (29.5 mm) and *C. procera* (28 mm) as compared to control. Then, effective phytopowders were applied under greenhouse and field conditions @ 5.0, 7.5 and 10.0% against citrus canker. Combination of *X. strumarium* + *S. baryosma* showed minimum disease severity (22%) followed by *X. strumarium* (26%), *X. strumarium* + *S. fruticosa* (27%), *S. fruticosa* (27%), *X. strumarium* + *S. baryosma* (27%), and *S. baryosma* (29%) as compared to control. While in field experiment, the combination of *X. strumarium* + S. *fruticosa* showed significant results with minimum disease severity (32%) followed by *S. fruticosa* + *S. baryosma* (32%), *X. strumarium* + *S. baryosma* (33%), *S. baryosma* (35%), *X. strumarium* (36%) and *S. fruticosa* (36%) as compared to control. It is concluded that application of *X. strumarium* + *S. baryosma* phytopowders will be helpful for farmers to combat citrus canker.

Keywords: Citrus Canker; eco-friendly management; phytoextracts; *Xac*

ABSTRAK

Sitrus ialah salah satu tanaman buah-buahan yang paling penting di seluruh dunia. Ia adalah punca yang kaya dengan antioksidan dan vitamin C. Kanker sitrus merupakan ancaman yang berpotensi kepada pengeluaran yang berjaya. Dalam kajian ini, sepuluh fitoserbuk padang pasir termasuk *Xanthium strumarium, Dipterygium galucun, Leptadenia pyrotechnica, Haloxylon recurvum, Suaeda fruticosa, Salsola baryosma, Citrulus colocynthis, Abutilon indicum, , Aerva javanica* dan *Calotropis procera* pada kepekatan berbeza (5.0, 7.5 dan 10.0%) telah dinilai dalam keadaan *in vitro* terhadap *Xanthomonas citri* pv. *citri*. Antara semua fitoserbuk, *X. strumarium* dan *S. fruticosa* menunjukkan zon perencatan maksimum (40 mm) diikuti oleh *S. baryosma* (38.50 mm) *C. colocynthis* (37 mm), *Abutilon indicum,* (34 mm), *H. recurvum* (32 mm), *D. galucun* (30.5 mm), *A. javanica,* (29.50 mm), *L. pyrotechnica* (29.5 mm) dan *C. procera* (28 mm) berbanding kawalan. Kemudian, fitoserbuk yang berkesan telah digunakan di bawah keadaan rumah hijau dan ladang @ 5.0, 7.5 dan 10.0% terhadap kanker sitrus. Gabungan *X. strumarium* + *S. baryosma* menunjukkan tahap keterukan penyakit minimum (22%) diikuti oleh *X. strumarium* (26%), *X. strumarium* + *S. fruticosa* (27%), *S. fruticosa* (27%), *X. strumarium* + *S. baryosma* (27%), *S. baryosma* (29%) berbanding kawalan. Manakala dalam uji kaji lapangan, gabungan *X. strumarium* + *S. fruticosa* menunjukkan keputusan yang ketara dengan keterukan penyakit minimum (32%) diikuti oleh *S. fruticosa* + *S. baryosma* (32%), *X. strumarium* + *S. baryosma* (33%), *S. baryosma* (35%), *X. strumarium* (36%) dan *S. fruticosa* (36%) berbanding kawalan. Disimpulkan bahawa penggunaan fitoserbuk *X. strumarium* + *S. baryosma* akan membantu petani untuk memerangi kanker sitrus.

Kata kunci: Ekstrakfito; kanker sitrus; pengurusan mesra alam; Xac

Introduction

Citrus is one of the most important fruit crops, grown in different areas of the world including Pakistan (Rehman et al. 2020). China, Brazil, USA, India, Mexico, and Spain are world's leading citrus growing countries (FAO 2017), while in Pakistan, it ranked 1st among all fruits in terms of production (Farooq 2014). In Pakistan, it is cultivated on an area of 206 thousand hectares with 2.2 million tons production (Memon 2017). Worldwide annual production of citrus is estimated to be more than 120 million tons. From which, about 50 percent is wasted in the form of citrus peel (Yadav & Sharma 2018). Citrus is a rich source of essential minerals, fibers, vitamins and bioactive phytochemicals such as alkaloids, nitrogenous compounds, polyphenolics and carotenoids (Mahato et al. 2018). However, its production is facing many biotic and abiotic problems worldwide (Jia & Wang 2020). Among biotic issues, citrus canker caused by *Xanthomonas citri* subsp. *citri* (*Xcc*), is a potential threat to citrus industry and is the primary cause for reducing quality and quantity of the produce (Daungfu et al. 2019). Citrus canker not only reduces cosmetic value of the fruit but also decreases its yield and market value (Lanza et al. 2019).

When *Xcc* attacks on citrus plants, it produces necrotic lesions (2-10 mm diameter) on leaves, fruits, and twigs, which results in defoliation and tree decline. After specific pause of time, these lesions become twisted on leaf, stem, and fruit. Later on, borders of these lesions become watery with yellow hollow and their size depends upon degree of resistance and susceptibility of the plant as well as on the virulence of the pathogens (Cernadas & Beneditti 2009). Susceptibility of the host plant towards canker disease increases due to production of 'Xanthan' as it overwhelms the defense mechanism of the citrus plant by changing physiology, growth and development along with dispersal of pathogen (Afroz et al. 2013). From season to season, *Xcc* survives in the previously infected twigs, tissues or debris and acts as a primary source of inoculum (Dewdney et al. 2016; Mansfield et al. 2012; Villamizar & Caicedo 2019).

Numerous chemicals are in practice to control citrus canker but due to unwise application of chemicals and their residual effects, crafting health issues in human beings, animals, and are not ecofriendly (Riera et al. 2018). Excessive use of chemicals (especially Cu based) against *Xcc* also produces resistance in bacteria (Young et al. 2017). This thing enforces researchers and scientists to find out some alternative to chemicals to overcome citrus canker. In this scenario, use of phytoextracts is the most appropriate option to manage plant diseases as they contain biological active compounds which act as bactericides, fungicides, nematicides or insecticides which are ecofriendly and are safer for human health. Among plants, desert plants are considered as origin of significant bioactive compounds, owing to existence of hazardous conditions. Under adverse conditions, plants produce high amount of reactant oxygen species (ROS) which induces oxidative stress to the plant cells. Compounds that are produced in plant cells are non-enzymatic and enzymatic antioxidants to prevent such ROS-generated influences (Ben Yakoub et al. 2018; Radwan et al. 2020). The plants which are present in deserts are the key foundation of antioxidants that store bioactive compounds in their tissues such as roots, flowers, leaves, seeds, and base (Al-Faifi 2019). Antibacterial agents like enhydrin is present in plants with higher antibacterial activity, but the optimal effectiveness of medicinal plants may not be due to only one main active compound (Choi et al. 2010). Leaves of desert plants are widely used for the herbal remedies because they are abundant in bioactive compounds (carotenoids and flavonoids) (Ahmad et al. 2014; Azimbaeva et al. 2020). The future looks bright for identifying new classes of pesticides (cost effective and safe) from plant extracts to replace the synthetic chemicals, used to control citrus canker (Danahap & Wonang 2016). So, the purpose of the present study was to find out the effective desert phyto-powder to manage citrus canker.

Materials and Methods

Isolation of bacterium associated with citrus canker

To isolate *Xcc* (by streaking method), citrus leaves exhibiting characteristic symptoms of citrus canker, were taken in brown paper bags (10×12 cm), and brought to Citrus Pathology Laboratory. For isolation of *Xcc*, nutrient agar (NA) media was prepared by adding Peptone 5 g, Glucose 2.5 g, Beef extract 3 g and agar 15 g in glass bottle and making 1 L volume by adding distilled water and sterilized the NA media in an autoclave (RTA 85, Robus United Kingdom) at 121 °C and 15 lbs pressure for 15 min (Hemraj et al. 2013). Infected portion of leaves were cut into pieces of 5-7 mm along with some healthy portion of leaf. Then, these small pieces were surface sterilized by dipping them in 70% ethanol for 30 s. After this, three washings of distilled water were

given to pieces of leaves to remove excessive ethanol and then placed on sterilized filter paper to dry them.

NA (3-5 mL) was poured into 90 mm Petri dishes in the laminar air flow chamber (RTVL-1312, Robus United Kingdom) and after solidification of NA, small pieces of infected leaves were picked with the help of sterilized forceps, placed them on Petri dishes containing NA media, wrapped and incubated them at 25 °C for 24 h (Riaz et al. 2014). After 24 h of incubation, bacterial growth, in the form of yellow colonies was appeared on NA media around the infected portion of leaves. Then, it was further purified by transferring bacterial colony to another Petri dish containing NA through streaking method. Then again after 24-36 h of incubation, small single colonies of pure bacteria were appeared on Petri plates. Pure culture of bacteria was preserved in 50% glycerol and stored them at -80 °C in a refrigerator (ZLN-T 300 COMF) for further use.

IDENTIFICATION AND PATHOGENICITY OF *X. citri* subsp. *citri*

Identification of bacteria (appeared on NA plate) was done visually by observing morphological characters like colour, shape and type of bacterial colony, while biochemical tests were also performed like 3% KOH and Gram staining test (Holt et al. 2000; Mubeen et al. 2015). Koch's postulates were used for the confirmation of isolated *Xcc* (Juhasz et al. 2013). For this purpose, bacterial culture was added into test tube containing nutrient broth. This test tube was placed on shaker (RTSK-0300, Robus United Kingdom) at 200 rpm for 24 h to obtain maximum bacterial growth (Hoque et al. 2005). After shaking, it was centrifuged (H-200 NR, Kokusan, Japan) in controlled temperature of 4 °C at 10000 rpm for 10 min. Supernatant obtained after centrifugation was removed and the pellet remained was again centrifuged by suspending it in 10 mM $MgCl_2$ solution. Washed pellet obtained was re-suspended in 10 mM $MgCl_2$ solution for several times, and with the help of spectrophotometer (Hitachi U- 2001, model 121-003) concentration was adjusted at 10^8 CFU/mL (0.1OD) at 620 nm (Horque et al. 2005).

For pathogenicity test of *Xcc*, ten plants (one year old) of Kinnow were collected from nursery, Institute of Horticultural Sciences (HIS), University of Agriculture, Faisalabad (UAF), Pakistan and grown in pots (30 cm diameter) containing sandy clay soil. Initially, this soil was sterilized by using 1% formalin, by mixing it with the soil particles thoroughly, watered and covered it with water-soaked gunny bags in such a way, so that air cannot be passed through it for 3 days. Then, uncovered it and kept it in open environment for 3-4 days to eliminate fumes of formalin (Goswami et al. 2010). Pruning of plants was done for uniform flush and growth. All the plants were placed in sunlight, watered, and covered with polythene bags for at least 2 h. Before inoculation, optimum humid conditions and maximum stomatal opening were maintained to facilitate bacterial infection. By using syringe method (using a syringe needleless tuberculin of 1 cm^3), inoculation was done in greenhouse early in the morning (when maximum number of stomata were opened). About 2 µL of bacterial suspension was injected in plant leaf (on three areas of leaves, mid vein and each side) (Francis et al. 2010) while only distilled water was injected into leaves of control plants with three replications under Complete Randomized Design (CRD) and these plants were placed in greenhouse for two weeks (Amaral et al. 2010). Symptoms appeared after 15 days of artificial inoculation on the leaves of kinnow plants.

To confirm Koch's postulates, re-isolation of bacterium was done from artificially inoculated leaves. Obtained bacterium was confirmed on the basis of morphological traits with the original bacterium culture previously used for the inoculations. Bacteria which displayed similar colony pattern, like original culture, considered as pathogenic and used for further studies.

COLLECTION AND PREPARATION OF DESERT PHYTOPOWDERS

Ten plants including, *X. strumarium*, *D. galucun, L. pyrotechnica, H. recurvum, S. fruticosa, S. baryosma, Citrulus colocynthis, Abutilon indicum, A. javanica,* and *C. procera* were collected from desert area of Fort Abbas, Southern Punjab, Pakistan (Table 1). The plants were identified and confirmed by the researchers of Botany Department, The Islamia University, Bahawalpur and further authenticated from the data published in Flora of Pakistan (http://www.eFloras.org). The stem and leaves of plants were collected, cleaned, and dried under shade for seven days. Then, plants were sun dried for three days, chopped into small pieces and grinded with the help of pestle and mortar until converted into powdered form. Then, this powder was further grinded in the electrical grinder (DY89-11). After this, powder of each desert plant was passed through muslin cloth to obtain a very fine powder (2 mm). The fine powder was kept in airtight container at 4 °C to avoid contamination. For preparation

TABLE 1. Desert plants with local and English names

Sr. No.	Scientific name	Local name	English name
1	*Xanthium strumarium* L.	Pahari londra	Cocklebur
2	*Suaeda fruticosa* (L.) Forssk	Rigit	Seablight
3	*Salsola baryosma* (Roem. & Schult.) Dandy.	Lana	Ressal
4	*Dipterygium glaucum*	Safrawi	Safrawi
5	*Citrulus colocynthis* (L.) Schrad.	Tuma	Desert gourd
6	*Haloxylon recurvum* (Bunge ex Fenzl).	Saxaul	Saksaul
7	*Aerva javanica* (Burm.f.) Shult.	Bue	Desert cotton,
8	*Abutilon indicum* (Link) Sweet.	Peeli boti	Mallow
9	*Leptadenia pyrotechnica* (Forssk.) Decne.	Khipp	Broom bush.
10	*Calotropis procera* (Aiton) W.T.Aiton.	Aak	Apple of Sodom

of aqueous extracts, 10 g powder of each desert plant was mixed in sterilized distilled water separately in flasks and volume was adjusted up to 100 mL, and suspension of each desert plant was soaked for 50 hours and was filtered through Whatman filter paper (2 µm). Filtrate was saved and supernatant was discarded. Then, three concentrations (5.0, 7.5 and 10.0%) of each phyto-powder were prepared.

IN VITRO EVALUATION OF DESERT PHYTOPOWDERS AGAINST *Xanthomonas citri* subsp. *citri*

Desert phytopowders were evaluated through disc diffusion method against *X. citri* subsp. *citri* (*Xcc*) under laboratory conditions. For this purpose, autoclaved nutrient agar (NA) media was poured into Petri plates (9 cm) in the Laminar Flow Chamber (RTVL-1312, Robus United Kingdom)) and allowed to cool down for 5 min. After solidification of NA medium, streaking of *Xcc* was performed with the help of sterilized cotton swab by using 100 µL bacterial suspension. Three concentrations of plant extracts (5.0, 7.5 and 10.0%) were used against *Xcc*. Sterilized filter paper (10 mm) was dipped in the solution of phytopowder and labeled the Petri plates with permanent marker. Three replications of each concentration were used with one control treatment. Filter paper was dipped in the phytopowder solution with the help of sterilized forceps, picked and placed in the middle of Petri plates. Then, Petri plates were wrapped with wrapping sheet and placed them in an incubator (Heraeus) at 36 °C. Inhibition zone was measured with the help of digital vernier caliper (Model: EMS 62065-40) after 24, 48 and 72 h. The experiment was conducted three times for the cogency of the results.

EXPOSURE OF SECONDARY METABOLITES IN AQUEOUS DESERT PHYTOPOWDERS

Aqueous desert phytopowders (10% v/w) of those plants that expressed maximum inhibition zone against growth of *Xcc* under *in vitro* conditions were prepared for the presence of antibacterial metabolites in them. For detection of alkaloid, 1% HCl (1.5 mL) was mixed with aqueous desert phytopowder (2 mL) in a test tube and was heated in a water bath for few minutes. and then 5-6 drops of Mayer's reagent were added to it. Advent of orange colour precipitate (ppt) exhibited the presence of alkaloid (Rasool et al. 2010). For the detection of flavonoid, lead acetate (few drops) was mixed in 2 mL of phytopowders. Appearance of yellow ppt. expressed the existence of flavonoids (Salhan et al. 2011). Similarly, for the existence of tannins and saponins in aqueous extracts, ferric chloride (few drops) was added in 5 mL of phytopowder. Formation of black ppt. indicated the presence of tannins, while saponins was detected, by adding 5 mL of distilled water in 2 mL of desert phytopowder and then agitated this mixture for 3 min, which resulted in frothing, that confirmed the presence

of saponins (Saidulu et al. 2014). Glycosides were determined in aqueous extract by following the protocol of Sachin et al. (2011).

APPRAISAL OF AQUEOUS DESERT PHYTO-POWDERS AGAINST CITRUS CANKER DISEASE UNDER GREENHOUSE CONDITIONS

Desert phytopowders, which exhibited maximum inhibition zone against *Xcc,* were evaluated under greenhouse and field conditions against citrus canker on susceptible citrus cultivar (Kinnow). For inoculation, inoculum was prepared @ 1×10^8 CFU per mL of water by using spectrophotometer (Hitachi U-2001, model 121-003) and inoculation was done early in the morning by using hypodermic syringe method. For this purpose, *Xcc* suspension was taken in a syringe of 23Gx 1" (0.60 - 25.0 mm) and injected into the veins along the midrib of citrus leaves (Klement 1963). After five days of inoculation, 5 mL of plant extracts was taken from each concentration (10, 20 and 25% formulated aqueous suspension) and sprayed on the plants with the help of hand sprayer (Pressure: 0.25-0.45 MPA) under Completely Randomized Design (CRD). The data regarding disease incidence was recorded with seven days interval for three times (Bowers & Locke 2000). Each treatment was evaluated with three replications with one control. Control treatment was sprayed with only distilled water. The experiment was repeated three times for the accuracy of results.

ESTIMATION OF AQUEOUS PHYTOPOWDERS AGAINST CITRUS CANKER UNDER FIELD CONDITIONS

For field trial, one-year old plants were planted in already prepared field by maintaining P×P= 1.0 m and R×R= 1.5 m distances. Inoculation of *Xcc* was done in the morning time and 5 mL of plant extract from each concentration (10, 20 and 25%) was applied while control plants were sprayed with distilled water. The inoculated plants were observed on daily basis and data was recorded after 10, 20, and 30 days interval. This experiment was conducted under RCBD with three replications. Disease incidence was measured by using following formula.

$$\text{Disease Incidence\%} = \frac{\text{No of infected plants}}{\text{Total No of plants observed}} \times 100$$

STATISTICAL ANALYSIS

At 0.05% probability level, means of different treatments were separated by using Fischer's protected least significant difference (LSD). ANOVA table, interactions between treatments × concentrations and Treatments × days were performed through SAS/STAT statistical software (Steel 1997).

RESULTS

IN-VITRO EVALUATION OF DESERT PHYTOPOWDERS AGAINST *X. citri* subsp. *citri*

ANOVA table indicated that all treatments and their interactions except T × C × D showed significant response against *Xac* (Table 2). Among all the treatments, *X. strumarium* showed maximum inhibition zone (39.93 mm), followed by *S. fruticosa,* (39.55 mm), *S. baryosma,* (38.50 mm), *Citrulus colocynthis* (36.58 mm), *Abutilon indicum* (33.99 mm), *H. recurveum* (31.54 mm), *D. galucun* (30.47 mm), *A. javanica* (29.50 mm), *L. pyrotechnica* (29.37 mm) and *C. procera* (27.73 mm) as compared to control (Figure 1), while interaction between treatments (T) and concentrations (C) indicated that *X. strumarium* expressed maximum inhibition zone (35.86 mm, 40.48 mm, 43.47 mm) followed by *S. fruticosa,* (36.13 mm, 38.75 mm, 41.38 mm), *S. baryosma,* (34.09, 36.21, 38.65 mm), *Citrulus colocynthis* (31.41 mm, 33.49 mm, 36.62 mm), *Abutilon indicum* (28.70 mm, 31.83 mm, 35.44 mm), *H. recurveum* (27.66, 31.95, 35.00), *D. galucun* (25.10 mm, 27.99 mm, 31.11 mm) *A. javanica* (23.50, 26.99, 30.11 mm), *L. pyrotechnica* (26.11 mm, 28.80 mm, 33.18 mm) and *C. procera* (23.67 mm, 26.76 mm, 29.22 mm) at (5.0, 7.5, 10.0%) concentrations respectively as compared to control (Figure 2).

In case of the interaction between treatments and days, all the treatments showed significant results (Table 2). After 24 hours, *X. strumarium* showed (38.00 mm) inhibition zone followed by *S. fruticosa,* (37.00 mm), *S. baryosma,* (36.00 mm), *Citrulus colocynthis* (36.00 mm), *Abutilon indicum* (31.00 mm), *H. recurveum* (28.00 mm), *D. galucun* (29.00 mm), *A. javanica* (28.00 mm), *L. pyrotechnica* (27.00 mm) and *C. procera* (24.00 mm), respectively. While after 48 hours, *X. strumarium* (40.00 mm), *S. fruticosa* (39.00 mm), *S. baryosma* (38.00 mm), *Citrulus colocynthis* (38.00 mm), *Abutilon indicum* (33.00 mm), *H. recurveum* (30.00 mm), *D. galucun* (30.00 mm), *A. javanica* (29.50 mm), *L. pyrotechnica* (28.00 mm), *C. procera* (26.00 mm), and after 72 hours, *X. strumarium* (42.00 mm), *S. fruticosa* (41.00 mm), *S. baryosma* (40.00 mm), *Citrulus colocynthis* (40.00 mm), *Abutilon indicum* (35.00 mm), *H. recurveum* (32.00 mm), *D. galucun* (31.00 mm), *A. javanica* (31.00 mm), *L. pyrotechnica* (29.00 mm), and *C. procera* (28.00 mm) inhibition zone, respectively, as compared to control (Figures 3 & 4).

TABLE 2. ANOVA for evaluation of different phytopowders against *Xanthomonas axonopodis* pv. *citri* under *in vitro* conditions

SOV	DF	SS	MS	F	P
Treatments (T)	10	33024.1	3302.41	2384.91	0.0000*
Concentrations (C)	2	3543.0	1771.49	1279.32	0.0000*
Days (D)	2	883.0	441.51	318.85	0.0000*
T × C	20	753.8	37.69	27.22	0.0000*
T × D	20	123.7	6.19	4.47	0.0000*
C × D	4	21.3	5.33	3.85	0.0049*
T × C × D	40	65.7	1.64	1.19	0.2230^{ns}
Error	198	274.2	1.38		
Total	296				

*= Significant, ns= Non-Significant

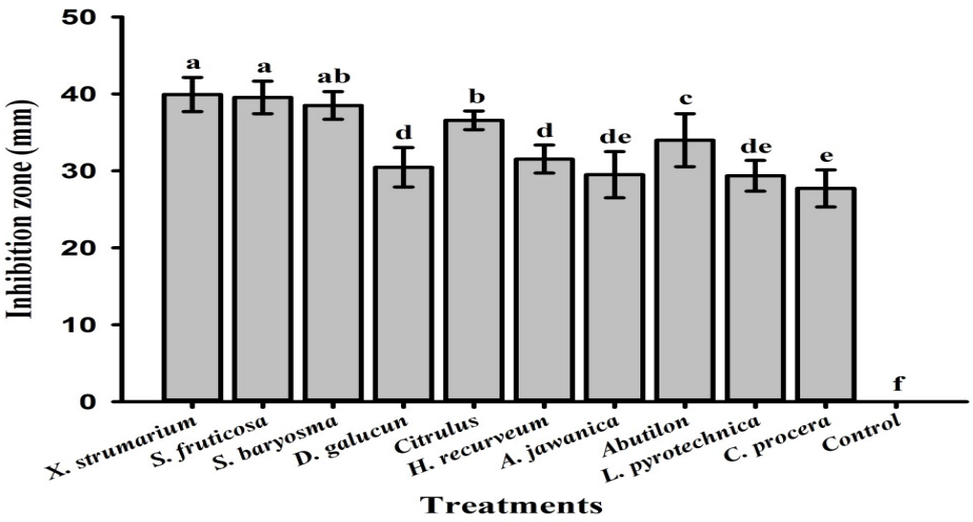

FIGURE 1. *In vitro* evaluation of desert phytopowder against *Xanthomonas axonopodis* pv. *citri*

EVALUATION OF DESERT PHYTOPOWDERS AGAINST CITRUS CANKER UNDER GREENHOUSE CONDITIONS

All treatments exhibited significant response against citrus canker disease under greenhouse conditions (Table 3). Among seven treatments, combination of *X. strumarium* + *S. baryosma* expressed minimum disease severity (22.38%) followed by *X. strumarium* + *S. fruticosa* (24.26%), *X. strumarium* (26.19%), *S. fruticosa* + *S. bartosma* (27.27%), *S. baryosma* (29.36%) and *S. fruticosa* (31.50%) as compared to control (Figure 5). While interaction between treatments and days, combination of *X. strumarium* + *S. baryosma* exhibited superior results with disease severity (25.16%, 22.60%, 19.40%) after 7th 14th and 21st days, respectively, followed

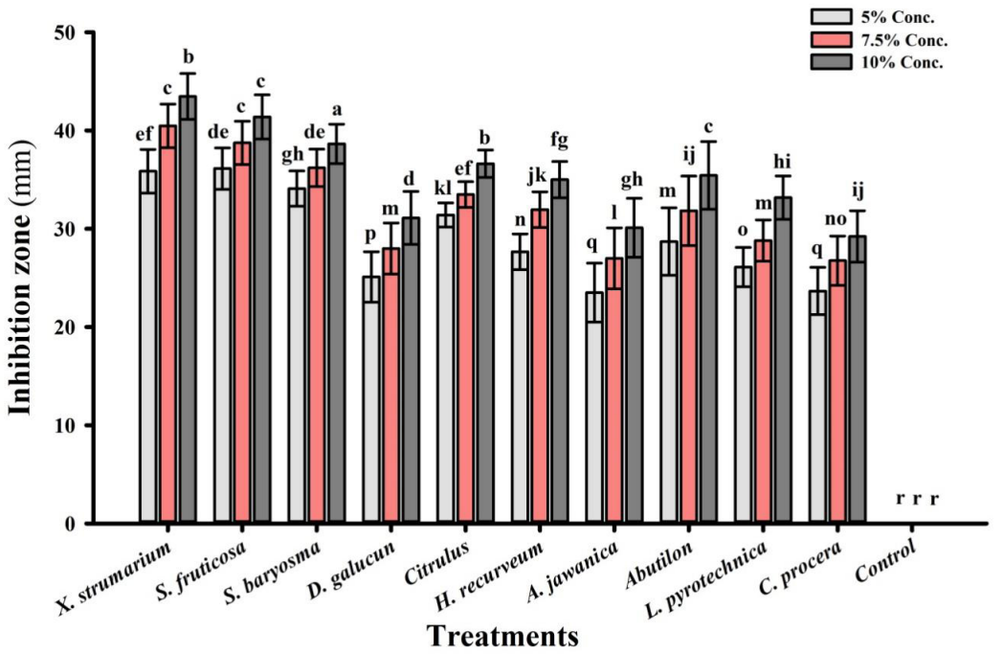

FIGURE 2. Interaction between treatments and concentration on bacterial growth under laboratory conditions

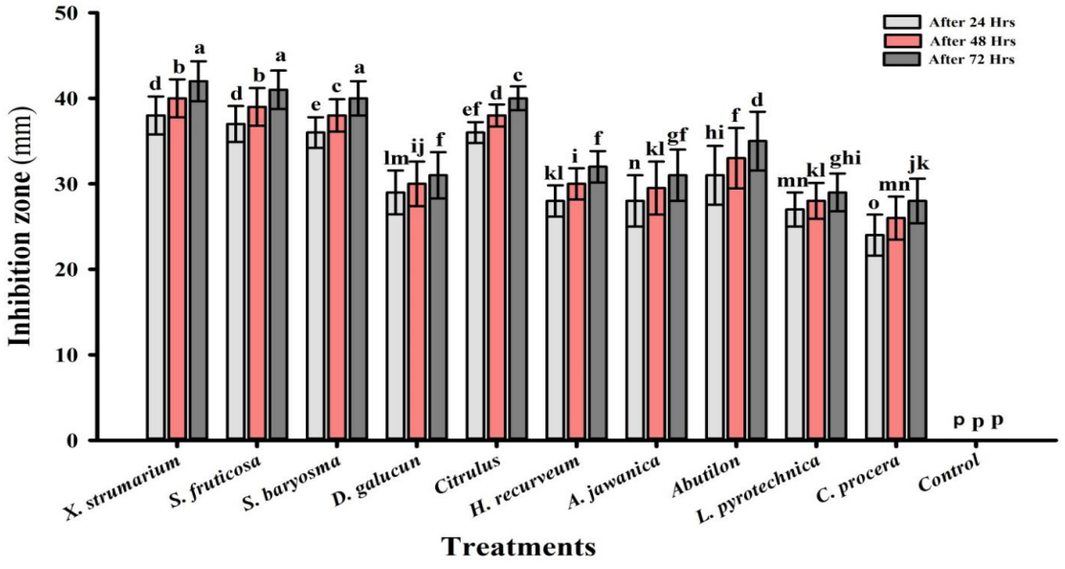

FIGURE 3. Interaction between treatments and time (hours) on bacterial growth under laboratory

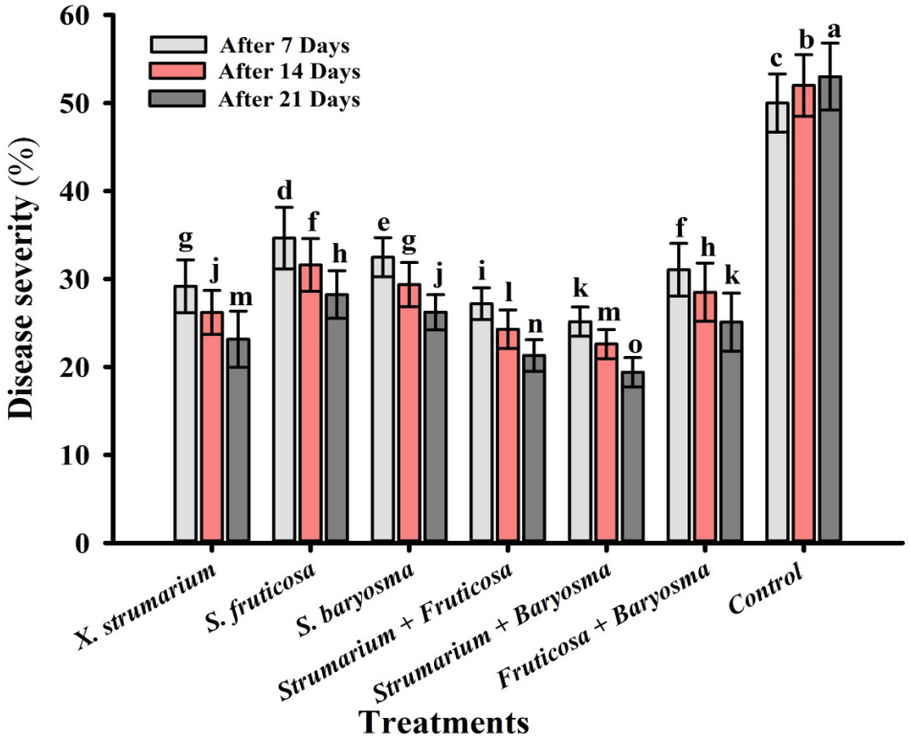

FIGURE 4. Inhibition zone of *Xanthium strumarium* against *Xanthomonas citri* pv. *citri*

by *X. strumarium* + *S. fruticosa* (27.19 %, 24.30 %, 21.30 %), *X. strumarium* (29.19%, 26.21%, 23.16%), *S. fruticosa* + *S. baryosma* (31.06%, 28.50%, 19.40%), *S. baryosma* (32.48%, 29.37%, 26.23%) and *S. fruticosa* (34.65%, 31.61%, 28.23%) as compared to control (Figure 6).

TABLE 3. ANOVA for evaluation of different phytopowders against citrus canker disease under greenhouse conditions

SOV	DF	SS	MS	F	P
Treatments (T)	6	4595.53	765.922	942.57	0.0000*
Days (D)	2	283.07	141.533	174.18	0.0000*
T × D	12	47.90	3.992	4.91	0.0001*
Error	42	34.13	0.813		
Total	62	4960.63			

*= Significant

EVALUATION OF DESERT PHYTOPOWDERS AGAINST CITRUS CANKER UNDER FIELD CONDITION

Under field conditions all treatments showed significant response against citrus canker (Table 4). Among all treatments, combination of *X. strumarium* + *S. baryosma* exhibited excellent results with minimum disease severity (26.34%) followed by *X. strumarium* + *S. fruticosa* (28.22%), *X. strumarium* (29.56%), *S. baryosma* (31.54%), *S. fruticosa* + *S. baryosma* (31.28%) and *S. fruticosa* (35.30%) as compared to control (Figure 7).

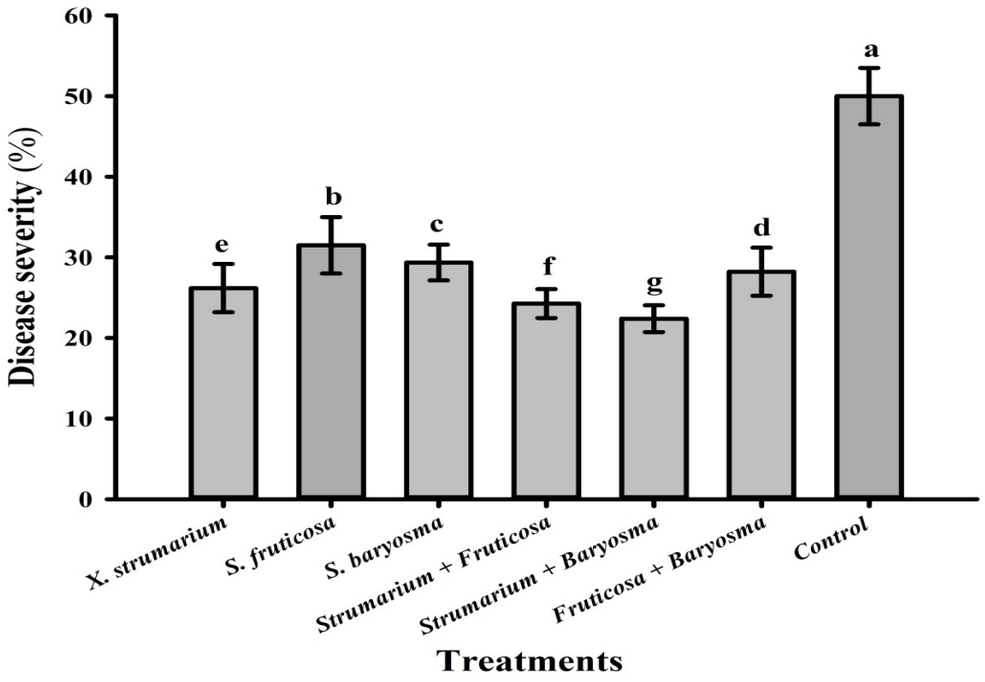

FIGURE 5. Assessment of phytopowders on the severity of citrus canker under greenhouse conditions

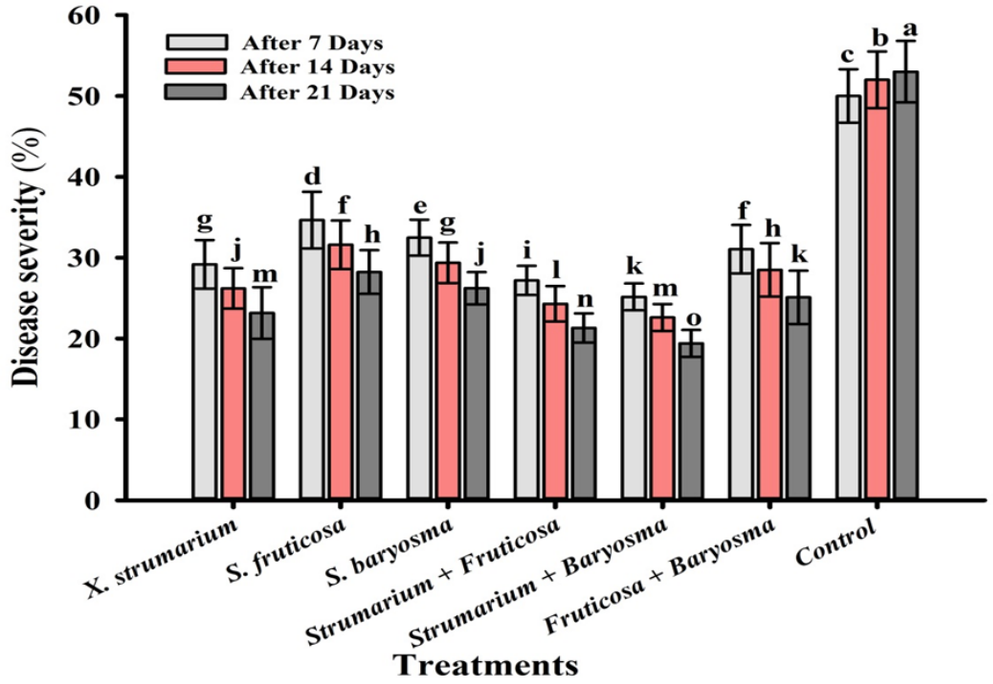

FIGURE 6. Evaluation of interaction between T & D against citrus canker under greenhouse conditions

Interaction between treatments and days showed that combination of *X. strumarium* + *S. baryosma* showed indomitable results with minimum disease severity (29.23%, 26.63%, 23.16%) after 7th, 14th and 21st days, respectively, followed by *X. strumarium* + *S. fruticosa* (31.16%, 28.16%, 25.33%), *X. strumarium* (32.30%, 29.16%, 27.23%), *S. fruticosa* + *S. baryosma* (34.33 %, 31.16 %, 28.36%), *S. baryosma* (35.00%, 31.43%, 28.16%), and *S. fruticosa* (38.16%, 35.46%, 32.26%) as compared to control (Figure 8).

TABLE 4. ANOVA for evaluation of different phytopowders against citrus canker disease under field conditions

SOV	DF	SS	MS	F	P
Treatments (T)	6	5109.21	851.535	1074.44	0.0000*
Days (D)	2	242.91	121.456	153.25	0.0000*
T × D	12	84.85	7.071	8.92	0.0000*
Error	42	33.29	0.793		
Total	62	5470.26			

*= Significant

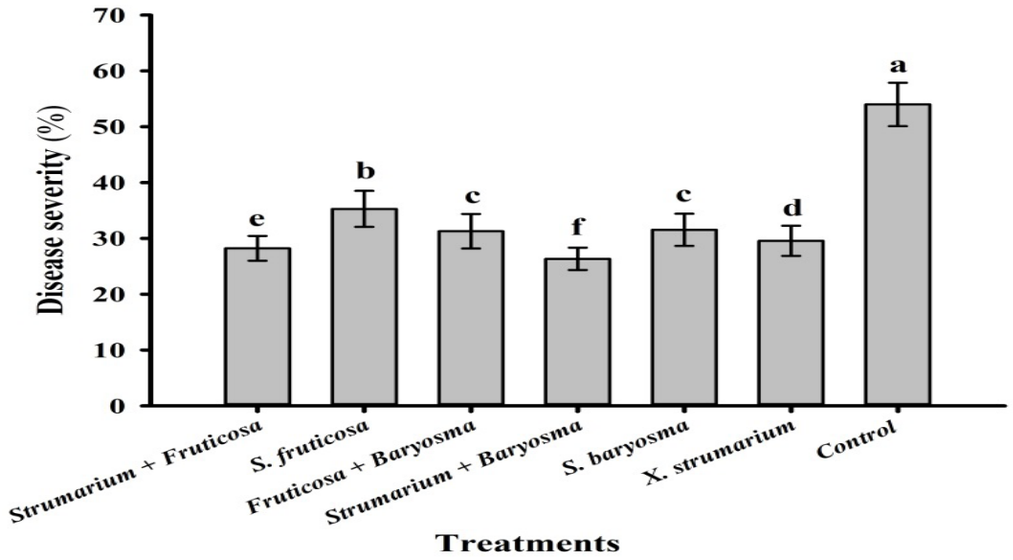

FIGURE 7. Impact of most effective desert phytopowders on disease severity against citrus canker under field conditions

DISCUSSIONS

Canker is a leading and hardly manageable threat to citrus industry (Nayem et al. 2017). Throughout the world, sudden and widespread of diseases are managed by synthetic chemicals but injudicious use of these chemicals creates environment pollution which is toxic to human and animals. Unremitting and undue use of these chemicals are towards *Xcc* creating resistance in pathogen which make difficult to manage citrus canker. The only safe and eco-friendly way to manage this disease, is the use of phytopowders, due to presence of antimicrobial substances in the plants (Prakash &

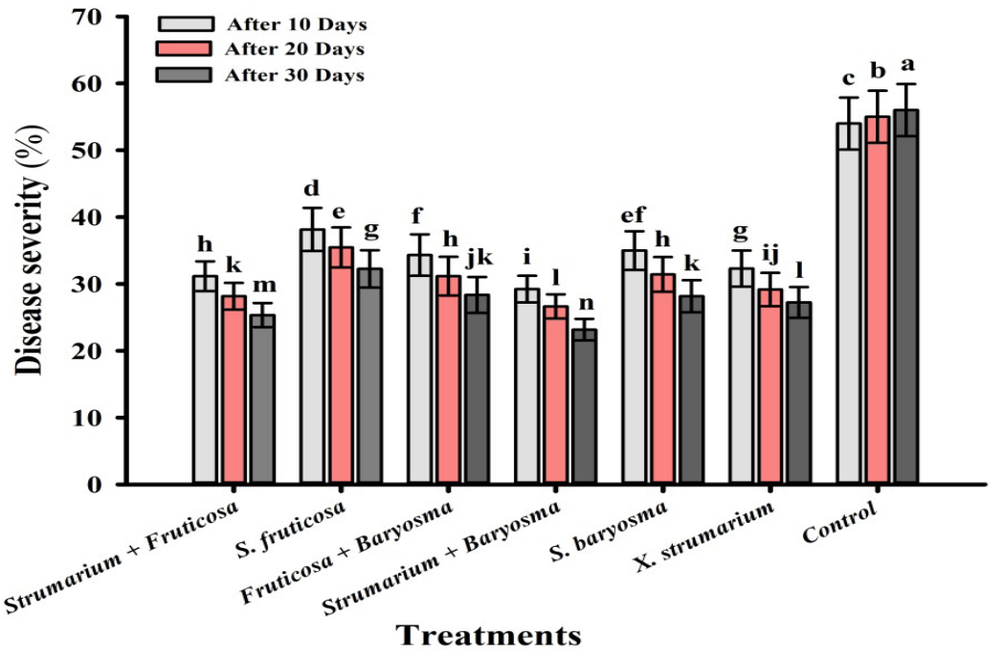

FIGURE 8. Impact of interaction between treatments and days against citrus canker under field conditions

Karmegam 2012) which are biodegradable, least toxic and economical phyto-pesticides (Asthana 2001). That is why in contemporary study, desert phytopowders were assessed against *Xcc*. Among all, phyto-powder of *X. strumarium* showed excellent results with maximum inhibition zone against *Xcc* followed by *S. fruticosa* and *S. baryosma*. Most effective phytopowders were applied under greenhouse and field conditions alone and in combination. *X. strumarium* alone and in combination with *S. baryosma* exhibited minimum disease severity. The results of current study are supported by Ebrahimi et al. (2020) who evaluated various weeds including *Amaranthus caudatus, Medicago sativa, Sorghum halepense, Xanthium strumarium, Portulaca oleracea, Avena ludoviciana, Plantago lanceolata,* and *Chenopodium album* under greenhouse conditions against citrus canker and concluded that extracts of weeds particularly *X. strumarium* expressed significant results towards citrus canker. Outcomes of the present study are in agreement with Jawalkar et al. (2020) who evaluated methanol extracts of *X. strumarium* against various pathogens and diseases and observed noticeable results against them.

Up till now more than 170 chemical constituents have been isolated and identified from *X. strumarium,* including phenylpropenoids, lignanoids, sesquiterpenoids, naphthoquinones coumarins, glycosides, thiazides, steroids, anthraquinones, flavonoids and other compounds. The primary compounds extracted from *X. strumarium* are carboxyatractyloside, caffeoylquinic acid, 1,5 di-caffeoylquinic acid and diterpene glucosides atractyloside (Van et al. 2020). The most essential chemical compounds of *X. strumarium* oil contain phenolic compounds such as β-caryophyllene (17.53 %), α-cadinol (6.66 %), spathulenol (6.09 %), limonene (5.66 %) (Parveen et al. 2017). These antimicrobial substances kill pathogens by different mechanisms such as disruption of cell membrane, coagulation of cell proteins, and interference with other vital functions. Alkaloids damage DNA and inhibit enzymes and saponins have been reported to react with the sterol component of cell membrane. Flavonoids usually affect proteins like extracellular proteins, cytoplasmic proteins, or enzymes (Khan et al. 2020). The active ingredient purified from Xanthium phloem has been known as SA (Salicylic acid). Dried powders of plants contain natural elicitor compounds which act as SAR activators to trigger the inactive defense systems of plants (Mitra & Paul 2017). Phytopowders alter the activities of antioxidant enzymes (SOD, POD, CAT), hydrogen peroxide, total phenols

and protein contents which are implicated in plant defense. The presence of phytohormones in botanical extracts influences the physiological process in a plant. Phytopowders contain considerable amounts of minerals which promotes nutrient uptake (which are responsible for activating defense system of the host plant towards plant pathogens) and improves the nutrient status and product quality of the plant (Yuniati et al. 2022).

Methanol extracts of *X. strumarium* are potential source of biocide and eco-friend disease controlling agents. It is also a strong candidate for further biological investigations (Ghahari et al. 2017). In another study, among the plants tested towards bacterial pathogen under *in vitro* conditions and the highest inhibition zone was expressed by methanol extract of *X. strumarium* Furthermore, extract of *X. strumarium* regulate respiratory burst oxidase homolog (RBOH) proteins that provide localized ROS bursts to regulate growth, developmental processes, and stress responses (Chapman et al. 2019). Moreover, current study is in line with Sharifi-Rad et al. (2015) who analyzed the essential oil of leaves of *X. strumarium* and found significant results in which the growth of *S. aureus, B. subtilis, K. pneumoniae,* and *P. aeruginosa* was suppressed. Similarly, Atiq et al. (2018) evaluated different plant extracts against *Xcc* and reported that extract of *A. indica* reduced the growth of bacteria under *in viv*o condition.

Conclusions

In the current study, a novel strategy has been used for management of citrus canker. Antibacterial potency of ten desert phytopowders has been evaluated. Among all phytopowders, *X. strumarium* and *S. fruticosa* expressed maximum biocidal potential towards *Xcc*. Therefore, these botanical extracts may be tested for protection of several other crops against a wide range of diseases.

Acknowledgements

We are highly thankful to Citrus Pathology Lab for providing research facilities under NRPU-HEC project# 6373. We are also thankful to Vice Chancellor, University of Agriculture, Faisalabad, Pakistan for their moral support for the completion of this study.

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