Article

Combined Effects of Biosolarization and *Brassica* Amendments on Survival of Biocontrol Agents and Inhibition of *Fusarium oxysporum*

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Abstract: Biocontrol agents (BCAs) added in the soil or applied to the seeds face many abiotic and biotic stress challenges. Only those BCAs that survive under harsh conditions perform well. Improving the survival of BCAs along with inhibiting the biotic stresses imposed by bacterial, fungal, and viral infections has been a major challenge in agriculture, especially in hot-arid climates. The present study aimed to evaluate the individual and combined effects of soil solarization and *Brassica* amendments on the survival of two biocontrol agents (BCAs), namely *Trichoderma harzianum* and *Aspergillus versicolor*, and on the reduction in a cumin wilt pathogen *Fusarium oxysporum* f. sp. *cumini* (*Foc*) in a field experiment conducted for two years under hot-arid climates. BCAs performed well in the solarized pots; it caused the maximum reduction in viable *F. oxysporum* propagules, significantly higher at 5 cm than at 15 cm of depth. *Brassica* amendment with BCAs caused a greater decrease in *F. oxysporum* propagules (95.7 to 96.7%) compared to a combination of BCAs and solarization (91.0 to 95.7%). Combining *T. harzianum* with *A. versicolor* increased the survival of *T. harzianum*, whereas integration with *Brassica* amendment could only improve the survival of *T. harzianum* at a depth of 5 cm and not at lower depths. The slightest decrease in *A. versicolor* population at high soil temperature was estimated when combined with *T. harzianum*. However, combining *A. versicolor* with *Brassica* amendment improved the survival of *A. versicolor* at high compared to low soil temperatures. Still, elevated soil temperature reduced the viable propagules. These studies demonstrate that both the native BCAs are compatible, and their integration with the *Brassica* amendment improves their survival and ability to reduce the population of cumin wilt pathogen. Thus, these BCAs with Brassica amendments can survive and perform well under hot-arid climates.

Keywords: *Aspergillus versicolor*; *Cuminum cyminum*; mustard residues; mustard oil cake; *Trichoderma harzianum*

1. Introduction

Cumin {*Cuminum cyminum* (L.)} is one of the oldest seed spices in the world. It is the second most popular spice in the world after black pepper. Today, it is mainly grown in Iran, Uzbekistan, Tajikistan, Turkey, Morocco, Egypt, India, Syria, Mexico, Bulgaria, Cyprus, and Chile. In Indian arid regions, cumin is grown in irrigated pockets on more than 0.5 million hectares during the winter [1,2]. The specific agro-climatic conditions favor cumin cultivation exclusively in the arid areas of Rajasthan and Gujarat states, India. India consumes a significant portion of cumin production domestically and exports cumin seeds to 70 countries worldwide [3]

Low organic matter, low microbial population, poor moisture retention capacity, and repeated cultivation of susceptible cumin genotypes to several pathogens have made the soil conducive to various threatening pathogens, which accounts for losses in the quantity and quality of the crop. Among multiple diseases of cumin, wilt caused by *Fusarium oxysporum* f. sp. *cumini* (Foc) is the most destructive disease, causing maximum yield losses, often up to 40% [4]. The wilt pathogen survives in hot-arid soil through specific resting structures, chlamydospores, and macroconidia. The population of these resting structures increases in soil with each successive round of cultivation [5,6]. After a few consecutive years of cultivation, the population density of pathogens increases in the soil to the extent where growers are compelled to abandon the cultivation of this economically valuable crop.

The implication of chemical approaches to control wilt pathogens is not helpful for humans to consume cumin seeds directly, and pathogens develop resistance against applied chemical fungicides [4,6]. Therefore, in the past two decades, several nonchemical and ecofriendly approaches have been explored to reduce wilt pathogen density in the soil to a level where the cultivation of cumin is profitable [6,7]. Among these, soil solarization has been proposed as a promising and environment-friendly management approach that utilizes solar energy and, through direct thermal destructions, controls fungi, bacteria, nematodes, insects, and even weeds [8]. The approach can be executed by placing transparent plastic sheets over the production plots, which trap heat from solar radiation in the upper soil layers [6–8].

Various factors contribute to the overall effect of the soil solarization technique, including climatic factors, soil temperature, soil moisture, duration of heating, and mulching material [9]. Using biodegradable materials such as paraffin-wax emulsion is also a better choice for mulching material [10,11]. However, soil-solarization during crop-free periods is highly effective in reducing the population density of Foc in soil. The ample availability of high temperatures and solar irradiation in Indian arid zones increases cost-effectiveness and durability [11]. However, the soil-solarization method faces limitations with increasing soil depth. Therefore, attempts have been made worldwide to improve the effectiveness of soil solarization in controlling pathogens, particularly at lower soil depths, by combining it with other control strategies [12].

Biosolarization is one such combining approach where the heating effect of solarization in combination with biocontrol amendments enhances the impact of soil-solarization in reducing pathogens load [13]. Various organic modifications followed by soil solarization have been reported to increase beneficial microbe interaction in the soil and induce resistance in the host plants [13]. Combining cruciferous residues with soil solarization significantly improved the control of the *F. oxysporum* level above that of soil solarization alone. This was attributed to the antifungal volatiles released from cruciferous residues during solarization and enzymatic hydrolysis of glucosinolates [14]. Glucosinolates are present in Cruciferous plants in various quantities; it produces sulfur compounds with antimicrobial activities [15,16]. *Brassica* species were also found with potent amendment capabilities by virtue of the presence of compounds such as allyl-isothiocyanates in their leaf extracts with antifungal activities [17,18]. Biosolarization and chitin amendments have been reported to inactivate fungal pathogens, including *Fusarium oxysporum* f. sp. *cumini*. This inactivation was associated with the improved activity of chitinolytic microorganisms [19]. Soil biosolarization has emerged as a promising alternative to conventional soil sterilization

methods. The heating effect of the solarization method has been found in direct harmony with a concentration of volatiles and biotoxins released by biological amendments [20,21]. Lately, biocontrol agents have been exploited as biological amendments.

Combining biocontrol agent *Trichoderma harzianum* and solarization improves the control of *Sclerotium rolfsi* [22]. Similarly, the synergistic interaction between sublethal heating and *Talaromyces flavus* caused an increased mortality of microsclerotia of *Verticillium dahalia* [23]. Biocontrol agents such as a native strain of *T. harzianum* in *Brassica* residues amended soil-augmented wilt control [6]. A native strain of *Aspergillus versicolor* isolated from naturally heated residue-amended soil has also been found to reduce the population of *Foc* and wilt incidence [24]. This strain could withstand soil temperatures up to 62 °C and be multiplying more rapidly at 60 °C. It was observed that the population of this biocontrol agent increased more in heated *Brassica* residue-amended soil than in nonamended soil. Nutrients from decomposed residues and certain compounds isolated from heated cruciferous residues were reported to stimulate the germination of microorganisms, particularly species of *Aspergillus* and *Penicillium* [25]. These studies demonstrate that microbial antagonism might have operated concurrently or sequentially to eliminate viable propagules of soil-borne plant pathogens from residue-amended solarized soil. However, precise information on the combined effects of elevated soil temperatures, *Brassica* residues, and biocontrol agents on the survival of soil-borne plant pathogens is not available. The present study was conducted to determine the interactive effects of high soil temperature achieved by soil solarization, *Brassica* amendments, and the BCAs *A. versicolor* and *T. harzianum* on the survival of *Foc* as well as both the biocontrol agents in a typical hot-arid environment.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted at the Central Arid Zone Research Institute, Jodhpur (Rajasthan), India, during the hot summer days of the year 2011 (11–30 April) and 2012 (14 April–5 May). The loamy sand soil (85% and 8.9% clay, 5.5% silt) of the field had 0.03% total nitrogen, 0.25% organic carbon, 9 ppm of Olsen-Pat, a pH of 8.1, electrical conductivity of 0.88 dSm^{-1} (soil: water ratio 1: 2.5), bulk density of 1.56 g cm^{-3}, and 10.4% moisture-holding capacity (MHC).

2.2. Temperature and Associated Weather Parameters

During the experimental period, the climatological parameters of the maximum temperature ranged from 39.6 to 43.5 °C and 38.4 to 43.5 °C, the wind speed ranged from 1.9 to 10.9 km/h and 1.9 to 12.9 km/h, the sunshine varied from 8.5 to 11.6 h/d and 8.2 to 11.5 h/d, and the relative humidity ranged from 21 to 61% and 7 to 68% in 2011 and 2012, respectively. A decrease in temperature with an increase in soil depth in all, dry soil (DS), i.e., non-irrigated soil; wet soil (WS), i.e., irrigated soil; wet but solarized soil (W+SS) was recorded and is displayed in Figure 1.

During 20 days of experimentation, the temperature of DS at 5 cm of depth remained higher than 50 °C for the initial 14 days. It fluctuated between 35 and 53 °C in the later 6 days, while at 15 cm of depth, it remained near 40 °C for the initial 14 days and ranged from 33 to 40 °C in later days. The effect of irrigation on the temperature in dry heated soil recorded in terms of WS irrigation initially brought the temperature down to 40 °C at 5 cm and 34 °C at 15 cm of soil depth. The combined effect of heating and irrigation recorded as W + SS at 5 and 15 cm depths states a limitation of solarization at lower depths.
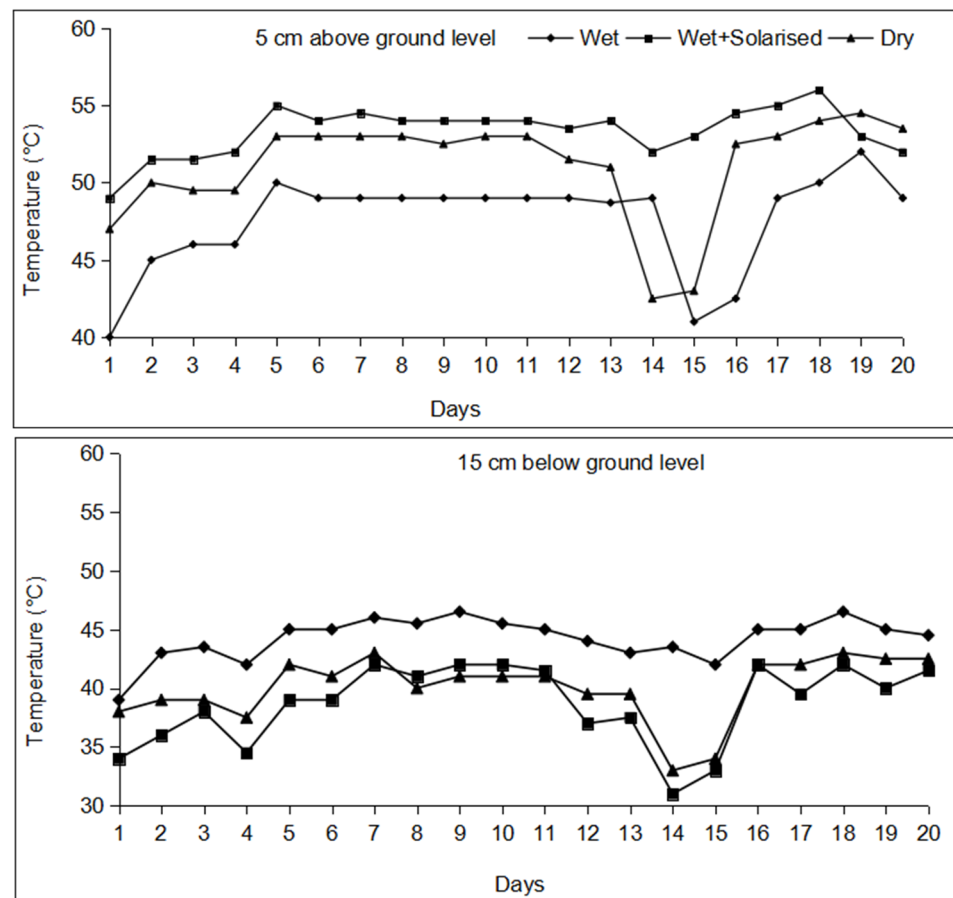
Figure 1. Fluctuations in soil temperature in dry soil (DS), wet soil (WS), and wet but solarized soil (W+SS) plot during 20 days of experimentation at both soil depths.

2.3. Pathogen Stabilization

The study on cumin wilt disease symptom development was undertaken on inoculated potted cumin plants. Identification of the *Fusarium oxysporum* f. sp. cumini (Foc) was confirmed based on morphological and pathological characteristics [4]. The confirmed pathogen inoculum was multiplied on corn-meal sand medium [4]. The Foc cultures were incubated for three weeks at 28 ± 2 °C with the daily shaking of flasks to obtain consistent growth. The inoculums prepared were added to the sterilized sand soil (2:1) potting mixture @ 10 percent (*w/w*) by mixing it with the upper layer of soil and allowing for 7 days to infect the soil. Cumin seeds were sown in the infected potting mixture after 7 days, once chlamydospores developed in the soil [1]. The seedlings transplanted in soil infected with the test pathogen were continuously monitored for symptom development until the plants were completely dead.

2.4. Pathogenicity Test

Foc was cultured on the artificial medium used as inoculum for cumin plants grown in pots and the control pot without inoculum for the pathogenicity test. The initial disease symptoms in Foc-inoculated seedlings developed after 10–15 days of inoculation (Figure 2). The pathogens were re-isolated from the inoculated seedlings and cultured using the same artificial media in the laboratory. The Foc colonies then obtained were compared with their original inoculated cultures [24]. The re-isolated pathogens resembled the original inoculated fungi in morphological, cultural, and pathogenic characteristics, satisfying Koch's pathogenicity postulates.

Figure 2. Pathogenicity test of isolated Foc on potted cumin seedlings.

2.5. Morphological Characterization and Identification of the Foc

Morphological characteristics of the causal pathogens were studied both on the host and the artificial potato dextrose agar culture medium. The pure culture of the Foc initially produced white colonies, which gradually turned into a light-pinkish color at the agar base and attained a growth of 90 mm in 7 days of incubation at 28 ± 2 °C [4,57]. When the collar region of the plant was cut vertically, the vascular bundles showed brownish discoloration [4]. Pathogen-inoculated plants developed initial symptoms as light yellow to brown discoloration of leaves followed by their shriveling, drooping, and ultimately wilting, leading to the death of the whole plant.

2.6. Inoculumpreparation

A strain of *Foc* isolated from the diseased roots of cumin was multiplied in bulk on a 5% corn-meals and (*w:w*) medium for 15 d at 30 ± 2 °C. The spores (conidia and chlamydospores) thus produced were passed through a mesh (53 μm) sieve to eliminate conidia [4]. The 1 kg of pre-sterilized infected soil left on the sieve was first examined under the microscope to confirm that it contains only chlamydospores of *Foc* and then mixed with 1.1 kg of the field soil to prepare *Foc*-infected soil inoculum, which was spread in iron trays and covered with a muslin cloth, then left for 10 days in bright sunlight (37–41 °C) for further stabilization before use in the experiment. The infected soil contained $3.4 \times 10^7/g$ chlamydospores as determined on the selective medium described in the 'Biological assays' section. *Aspergillus versicolor* isolated from the native soil of the arid region was multiplied in bulk on Potato Dextrose Broth. Fungal mats were harvested after 15 days on filter paper and blended for 5–6 min in 200 mL of sterilized distilled water. The individual suspension had a population of 10^{10} CFU/mL of *A. versicolor*. This homogenate culture was mixed in 500 g of field soil, divided into six equal sublots, and filled separately in nylon-net-covered tubular muslin pouches (9×9 cm). Likewise, *Trichoderma harzianum* was also multiplied using the specific selective medium.

2.7. Experimental Design

The combined effects of elevated soil temperature and *Brassica* amendment on the survival of *A. versicolor, T. harzianum,* and Foc were studied at 5 and 15 cm of soil depth in split-split plots in a randomized complete block design during both years. The inoculum of all the fungi mentioned above was multiplied in the laboratory as per the procedures.

2.7.1. Application of Biocontrol Agents and Pathogen Inoculum

One hundred fifty pocket-shaped small nylon-stitched pouches lined with muslin cloth were filled with 50 g of *A. versicolor*, *T. harzianum,* and Foc, alone and with different combinations in field soil.

2.7.2. Application of Brassica Amendment

Ground-up residues of 1% mustard pod straw + 0.02% mustard oil-cake (*Brassica* amendments) were amended in fungus-infected soil in the respective treatments where interactive effects of heat level and residues were to be ascertained.

Thus, the experiment comprised 11 treatments as follows: T1 = Foc, T2 = *T. harzianum*, T3 = *A. versicolor*, T4 = Foc + *T. harzianum*, T5 = *A. versicolor* + *T. harzianum*, T6 = Foc + *Brassica amendments* (BA), T7 = Foc + *T. harzianum* + BA, T8 = Foc + *A. versicolor* + BA, T9 = *T. harzianum* + BA, T10 = *A. versicolor* + BA, and T11 = *A. versicolor* + Foc.

2.7.3. Field Design

To set the experiment, the complete field was divided into three plots and named A, B, and C. Plot-A was marked for wet soil (WS) only, Plot-B was marked for wet + solarized soil (W + SS), and plot C represented dry soil (DS). An 8–9 cm high soil bund was erected on the border of each plot, and then each was subsectioned into three, representing replications, i.e., R1, R2, and R3. Each replication subsection was then divided into rows for 5 cm and 15 cm depth experimental requirements and each row contained 11 treatments. Plots-A and -B were irrigated plots, and irrigation was applied on 10 April 2011, and 13 April 2012, by flooding (45 cm depth) to field capacity (10.4% *w/w* or –0.003 Mpa). For solarization in Plot-B, a transparent polyethylene sheet (50 μm) was spread, i.e., mulching and boarders of these sheets were sealed by covering it with soil as an 8–9 cm high bund. Plot-C was kept nonirrigated and nonmulched. Soil temperatures were recorded daily from 11–30 April 2011 and 14 April–5 May 2012 in one representative plot of sets A, B, and C at 5 and 15 cm of depth at 14:00 h (Afternoon time) and 16:00 h (Evening time) by using an electronic data logger (Century Instruments, Chandigarh, India).

2.7.4. Experimentation Field Setup

In plots A and B replication, 22 pits were dug, 11 for each at 5 and 15 cm of depth. In replication subsections of Plot-C, only 6 pits were dug, 3 for each at 5 and 15 cm of depth. The distance between pits in one row was kept at 30 cm. Prepared pouches for biocontrol agents and pathogen and brassica amendment were then implicated in the field. The open end of each pouch was hand-stitched and tied with a nylon string and iron label, which was marked with the treatment number. Pouches according to treatment combinations were then buried in their respective pits in all plots. After filling, a soil sample of approximately 2 g was taken from each pouch to estimate each fungus' initial population of viable propagules on their respective media. All the pits were then covered with field soil so that the treatment-marked iron label with a part of the nylon string remained above the surface of each pit.

On 1 May 2011, and 6 May 2012, the pouches were retrieved gently from each pit, and adhering soil was removed. These were brought to the laboratory, and the soil of each pouch was transferred in separate polyethylene bags, air-dried, and processed to estimate viable propagules of *A. versicolor*, *T. harzianum,* and Foc on their respective media, as described in section 'Biological Assays'.

2.8. Biological Assays

The soil samples were air-dried and passed through a 2 mm sieve to quantitatively estimate microbes. The *A. versicolor* was calculated using the serial dilution technique on Martin's Rose Bengal Agar medium [25]. A serial dilution technique counted the colony-forming units (CFUs) of Foc on a modified peptone-PCNB (664 mg/L) medium [26]. A 10 g soil suspension in 90 mL of sterilized, de-ionized water was serially diluted twice (10^3). One milliliter of the final dilution was pipetted onto one plate. When no CFUs were detected at 10^3, these were estimated at 10^2 dilutions. White restricted colonies of Foc, which later turned pinkish, were easily distinguishable from others. The population of *Trichoderma* was estimated by the serial dilution technique on *Trichoderma* selective medium [20]. Six Petri dishes (9 cm) of each medium were used to enumerate each category from one soil sample. The means of 6 Petri dishes were considered a single estimation per replicate of each treatment. The survival proportion of *A. versicolor*, *T. harzianum,* and Foc propagules in each replication was computed by dividing the number of propagules. The completion of the experiment by the number of propagules at the beginning of the experiment and percentage reduction in viable propagules was calculated for each treatment.

2.9. Statistical Analysis

All the data of both years were pooled and then subjected to analysis of variance (ANOVA), and the treatment means were compared by LSD ($p = 0.05$). Each treatment's percentage of viable propagules was calculated after deducting from the initial viable population. Data on percent mortality and reduction in viable propagules were converted to angular transformed values before analysis [26]. The transformed data were analyzed following a split-plot design to identify the significance of the main effects of solarization, irrigation, amendments, soil depth, and their 2- and 3-factor interactions. Contrasts of interest were calculated for *A. versicolor*, *T. harzianum,* heat levels versus depth, and heat levels versus amendments. Fisher's LSD test was used for the mean separation of various treatments.

3. Results

3.1. Reduction in Foc Population

Combining biocontrol agents or Brassica amendments in all the treatments caused a more significant Foc propagules reduction than their lone application. Soil solarization further improved the reduction in Foc propagules, which was significantly ($p = 0.05$) greater at 5 cm compared to 15 cm of soil depth (Table 1). One summer irrigation in dry-heated soil (i.e., wet soil) caused a 28.3–36.7% reduction in Foc propagules within 20 days. Combining biocontrol agents improved this reduction at both soil depths, the decrease being more significant with *A. versicolor* than that achieved with *T. harzianum*. However, *Brassica* amendments with summer irrigation caused a reduction of 43.3–63.0% at both soil depths, which was significantly better than applying individual biocontrol agents in irrigated-only plots. The integration of *A. versicolor* with *Brassica* amendments reduced viable Foc propagules by 45.8–75%. This reduction was more remarkable than that achieved with the combination of *T. harzianum* with *Brassica*, i.e., 45–65%. The maximum decrease in viable Foc propagules was achieved with individual biocontrol agents and *Brassica* amendments at lower soil depths, i.e., 75%. Due to polyethylene mulching (wet but solarized soil), the elevated soil temperature drastically reduced the Foc population (87.9–96.5%) in all the treatment combinations at both soil depths during both years. Polyethylene mulching in nonamended soil reduced the Foc population by 92.8% at 5 cm and 87.9% at 15 cm of soil depth. Wet solarization with individual biocontrol agents at both depths reduced the Foc population by 91–93%, while the effect was enhanced to 93–96% when combined with Brassica. Therefore, this study supports the implication of integrative management strategies for reducing the population density of wilt pathogens, i.e., soil solarization in combination with biocontrol agent and biological amendments.

Table 1. Effect of amendments and summer irrigation on reducing *F. oxysporum f.* sp. *cumini* population (%) at different heating levels observed at two soil depths (5 cm and 15 cm).

Treatment *	Wet		Wet +Solarized		Dry soil	
	Soil Depth					
	5cm	15cm	5cm	15cm	5cm	15cm
Foc	36.7 (37.29)	28.3 (32.14)	92.8 (74.44)	87.9 (69.6)	3.9 (11.2)	2.5 (9.0)
Foc + *T. harzianum*	45.2 (42.13)	36.8 (37.23)	93.0 (77.30)	91.0 (72.54)	-	-
Foc + *A. versicolor*	46.3 (42.38)	36.9 (37.35)	93.7 (78.14)	91.3 (72.85)		
Foc + CR	63.0 (52.53)	43.3 (41.15)	95.2 (77.08)	92.4 (73.78)	-	-
Foc + *T. harzianum* + CR	65.0 (53.61)	45.2 (42.13)	96.0 (78.17)	93.2 (74.66)	-	-
Foc + *A. versicolor* + CR	75.0 (60.0)	45.8 (42.59)	96.7 (79.22)	94.0 (75.70)	-	-
Foc + *A. versicolor* + *T. harzianum*	51.0 (45.46)	38.5 (38.23)	95.3 (77.21)		-	-

LSD ($p = 0.05$) = Amendment: 0.16, Heat Level: 0.04, Amendment × Heat Level: 0.10, Depth: 0.03, Amendment × Depth: 0.09, Heat Level × Depth: 0.05. * Foc—*Fusarium oxysporum*; *T. harzianum*—*Trichoderma harzianum*; *A. versicolor*—*Aspergillus versicolor*; CR—Cruciferous residues (*Brassicas*) (1% mustard pod straw + 0.02% mustard oil-cake); angular-transformed values are in parentheses.

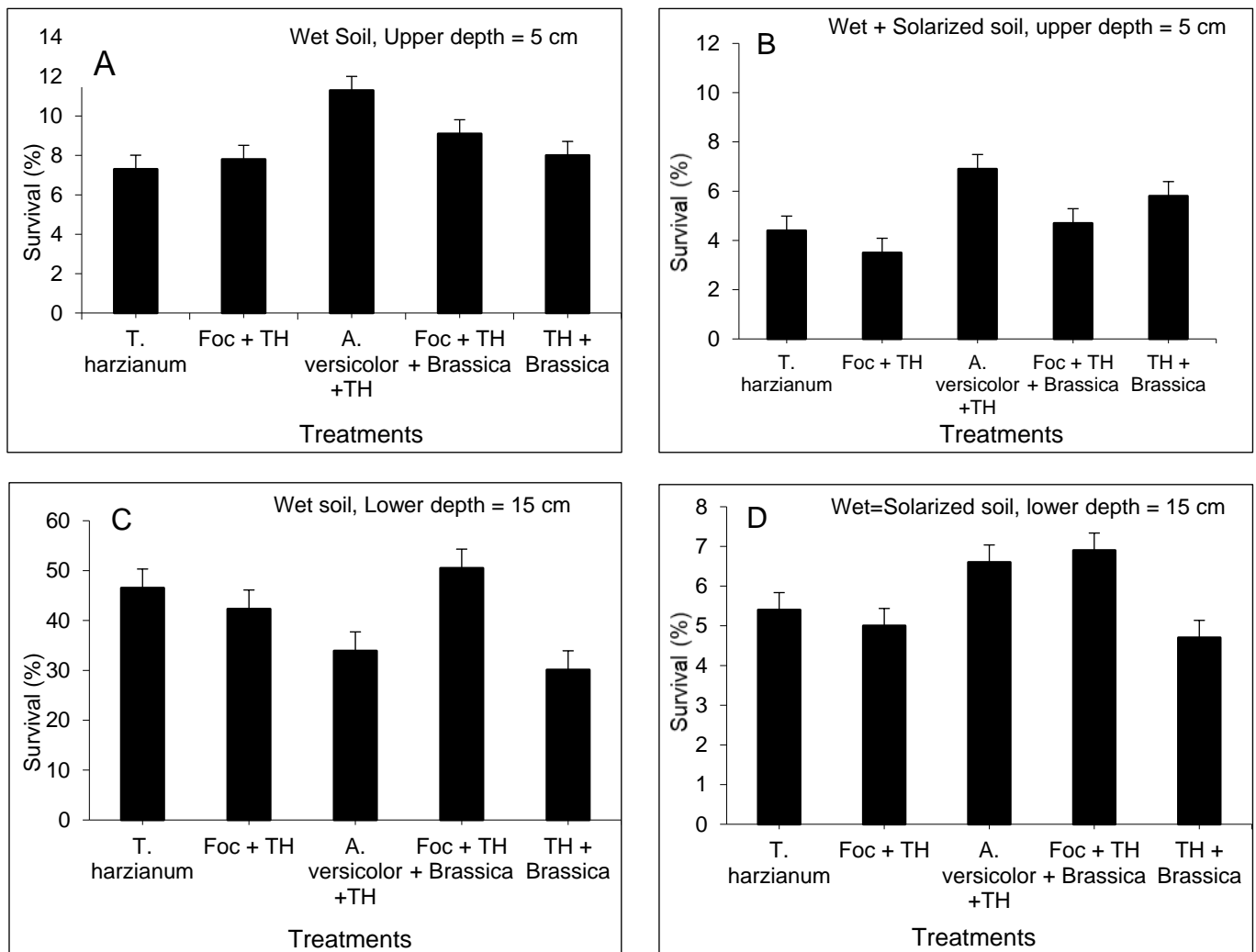### 3.2. Survival of T. harzianum Population

The population of *T. harzianum* survived by 7.3–46.5% at both soil depths in the irrigated-only (wet soil) plots during 20 days of the experiment (Figure 3). The survival of the biocontrol agent population in the *T. harzianum* + Foc combination followed the same trend, except that the survival level at the lower soil depth was significantly less than that achieved in the treatment with *T. harzianum* alone. Combining *T. harzianum* with *Brassica* produced similar results, but the survival was significantly less at lower soil depths. When *A. versicolor* was combined with *T. harzianum*, it favored the survival of *T. harzianum* propagules at 5 cm of soil depth, significantly higher than those recorded in any other treatment at the same depth, but the reverse occurred at lower soil depths. Integration of *T. harzianum*+ Foc+ *Brassica*, on the other hand, favored the survival of *T. harzianum* only at 15 cm of soil depth, where a 46.9% survival was estimated compared to a 9% survival at 5 cm of soil depth. Collectively, a lower soil depth favored the survival of *T. harzianum* in all combinations than in the upper soil layer.

Elevated soil temperatures in wet + solarized plots further reduced the survival of BCAs in all the treatment combinations, and the reduction levels were similar at both soil depths. The survival of the *T. harzianum* population ranged between 4.4 and 5.4% and 3.5 and 5.0% in the treatments having *T. harzianum* alone or combined with Foc propagules, respectively. Amending both biocontrol agents favored *T. harzianum* at both soil depths. *Brassica* amendments could also not support the survival of *T. harzianum* propagules to significant levels at both soil depths. The finding suggests irrigation favors germination and multiplication of *T. harzianum* propagules at deeper soil layers with moisture retention capacity. In contrast, irrigation with solarization significantly reduces the biocontrol agent's survival efficacy.

3.3. Survival of A. versicolor Population

The population of *A. versicolor* in all treatments under wet and wet + solarized plots was significantly higher than in *T. harzianum* treatment (Figure 4). *A. versicolor* has been reported to survive at elevated temperatures and could withstand solarization. The survival of *A. versicolor* was better in wet plots. Solarized plots indicated a significant role of heat in the germination and multiplication of biocontrol agents compared to wet-only plots.

Figure 3. Efficacy of Brassica amendments, irrigation, and soil solarization on survival of *Trichoderma harzianum* population. (**A**) Wet soil at 5 cm upper depth, (**B**) Wet = Solarized Soil at 5 cm upper depth, (**C**) Wet soil at 15 cm lower depth, (**D**) Wet = Solarized Soil at 15 cm lower depth. LSD (*p* = 0.05) Amendment: 0.15, Heat level: 0.07, Amendment vs. Heat level: 0.16, Depth: 0.04, Amendment vs. Depth: 0.17, Heat level vs. Depth: 0.06. Brassicas—1% mustard pod straw + 0.02% mustard oilcake.

In wet-only plots, the population of the bioagent survived by 62.5–65.2% at both soil depths. Combining *T. harzianum* with *A. versicolor* could not support *A.versicolor* survival to a significantly higher level, but combining it with *Brassica* improved survival at 5 cm of depth, i.e., 67.3%.

In a wet but solarized plot, the survival of *A. versicolor* improved to 60–80% at both soil depths. Furthermore, combining *T. harzianum* with *A. versicolor* under solarized conditions favored the survival of 84.9% propagules of *A. versicolor* at 5 cm and 67.3% survival at lower soil depths. The integration of *A. versicolor* with *Brassica* favored the survival of 55.8–82.4% propagules of *A. versicolor* at both soil depths. However, a combination of *A. versicolor* + *Brassica* + Foc or *A. versicolor* + Foc favored the viability of *A. versicolor* propagules at both soil depths to 50–60%. Analysis of variance showed that heat levels, amendments, depths, the interactions of amendments with heat levels, amendments with depth, and heat levels with depth were significant in all the pathogens, bioagents, and Brassica combinations.
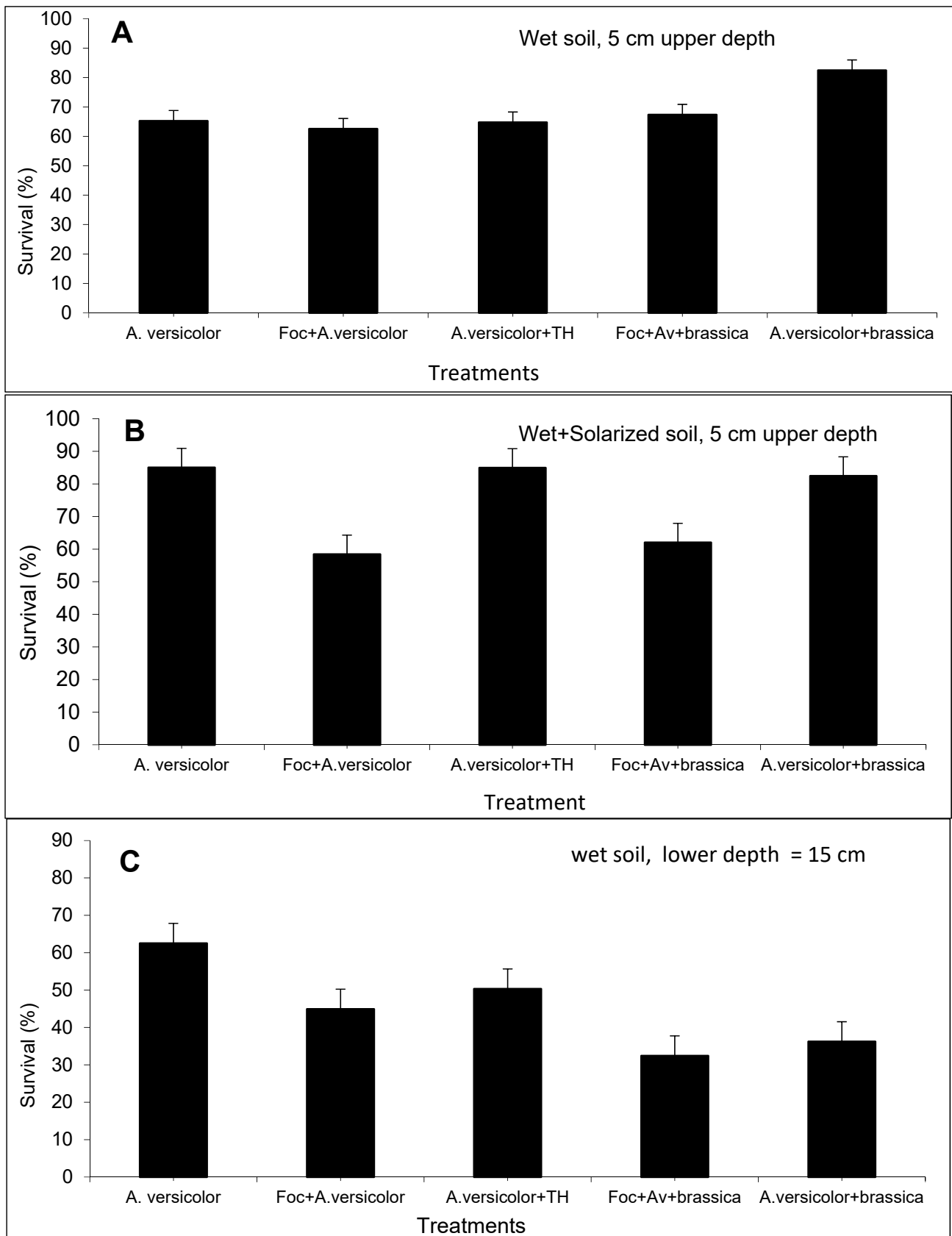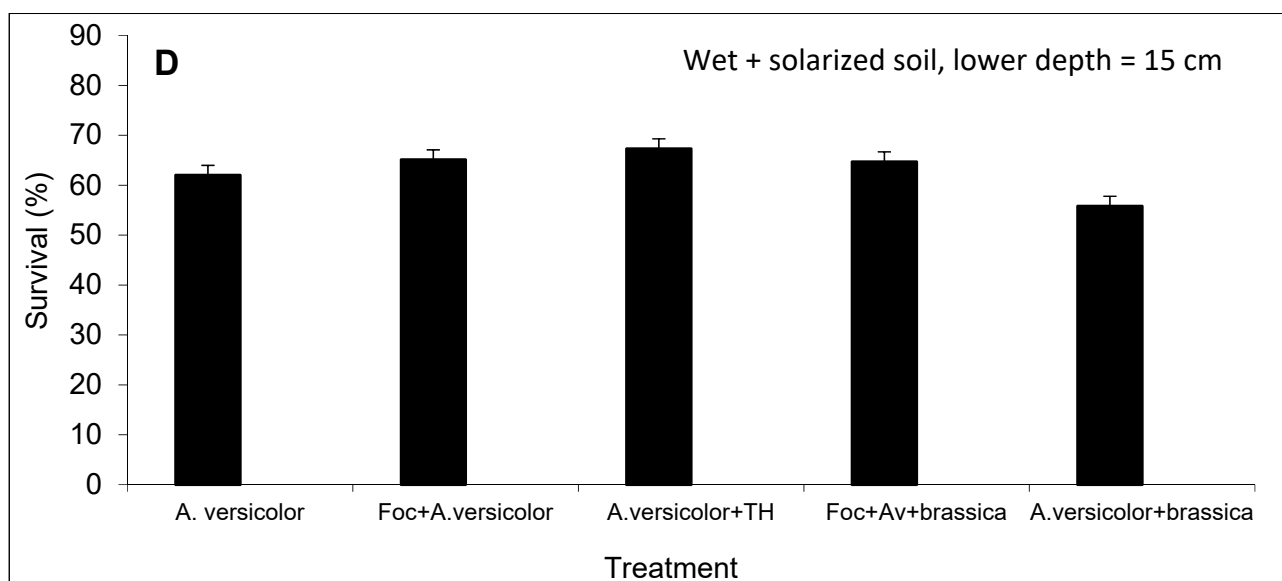
Figure 4. *Cont.*

Figure 4. Efficacy of *Brassica* amendments, irrigation, and soil solarization on survival of *Aspergillus versicolor* population. (**A**) Wet soil at 5 cm upper depth. (**B**) Wet=Solarized Soil at 5 cm upper depth. (**C**) Wet soil at 15 cm lower depth. (**D**) Wet=Solarized Soil at 15 cm lower depth. LSD (p = 0.05) Amendment: 0.03, Heat level: 0.02, Amendment vs. Heat level: 0.06, Depth: 0.02, Amendment vs. Depth: 0.05, Heat level vs. Depth: 0.03. *Brassicas*—1% mustard pod straw + 0.02% mustard oilcake.

4. Discussion

In combination with other control strategies, including soil amendments and biocontrol agents, soil solarization has been reported to enhance the overall effectiveness of the approach against pathogens and aid in improving crop production [27]. In the proposed study, field experiments conducted during hot summer days for two successive years recorded a significant reduction in Foc propagules using management strategies, including soil solarization, *Brassica* amendments, and biocontrol agents such as *Aspergillus versicolor* and *Trichoderma harzianum*, applied against cumin wilt pathogen. In dry control soil, a minimum reduction rate of only around 2–3% suggests the tendencies of Foc propagules to withstand high heat and the survival of Chlamydospores of Foc even at 60 °C [10]. In irrigated-only plots (wet soil), there were 36.7% and 28.3% reductions in Foc propagules at 5 and 15 cm of depth, respectively. Combining control approaches increases the maximum to 75% and 45% at respective depths. The possible control mechanism induced in irrigation-only soil at topsoil could be attributed to water dilution of the fungal population and concurrent microbial antagonism provided by amendments and biocontrol agents. At lower depths, moisture retention favors the germination of Chlamydospores, reducing the approach's efficacy [10,28]. The reduction achieved in Foc propagules in irrigated-only plots in the present study is in agreement with earlier findings [10,14,29]. Compared to irrigated-only and dry soil combinations, the maximum decrease in Foc propagules obtained with wet-solarized soil was amended with Brassica and individual biocontrol agents. These results are mainly attributed to soil moisture prevailing high temperatures, which directly correlates with the number of biotoxins and antimicrobial products released by bioamendments and the contribution of applied biocontrol agents [30]. Soil moisture provided by irrigation also affects the population density and sensitivity of chlamydospores in soil [18]. The maximum reduction rate for Foc propagules in wet but solarized soil with management combinations was 96.7% and 94% at 5 and 15 cm of soil depth, respectively. Varying degrees of reduction with soil depth may be attributed either to a higher dilution of fungistatic behavior of the soil at the minimum soil depth by irrigation than dilution of the fungus population at more profound levels, or to the limited soil-solarization method with increasing depths. Germination of chlamydospores is known to be vulnerable to

high temperatures, and thus with increasing depth, the effect of solarization-based control strategies reaches its limitation [31]. A remarkable reduction in Foc propagules, around 94% at lower depths, i.e., at 15 cm, in wet but solarized soil was observed than in wet-only soil at a similar depth, i.e., 45%. To a certain extent, the effect of water on the heat stability of fungal proteins is credited for these observations. Fungal proteins become susceptible to heat in the presence of water or upon hydration; the unfolding of proteins requires less heat energy; therefore, a greater rate of reduction in Foc propagules becomes possible even at lower depths in wet but solarized soil [32]. Soil moisture is a potent factor known to affect the efficacy of solarization, and few researchers have supported the idea of irrigation before solarization to balance the soil thermal equilibrium. Still, more progress is required on the subject to conclude the relationship between soil moisture and temperature [33,34]. The observed effect of *Brassica* amendment in reducing Foc was significantly higher than the effect of biocontrol agents alone at both soil depths, i.e., 5 and 15 cm, and solarization tends to enhance the release of the biotoxic-volatiles-aided higher reduction rate in wet but solarized soil. Soil temperature is affiliated with bioamendments' antimicrobial compounds and volatiles [11,17,35]. A combination of *Brassica* amendment with any two biocontrol agents shows a maximum reduction rate yet comparatively higher reduction with *A. versicolor* than *T. harzianum.* These results suggest that integrating solarization with biocontrol agents improves the control of soil pathogens [36]. A better control achieved with *A. versicolor* can also be attributed to its adaptability at high soil temperatures than *T. harzianum* [14]. This finding is significant in a hot-arid region where the soil temperature often reaches 50–55 °C in bare soil. Moreover, the results suggest that suppression could be achieved within 2 weeks, potentially making biosolarization more sustainable than chemical fumigation [37]. Resource-deficient growers who cannot afford to solarize their fields may achieve partial control of Foc by *Brassica* amendments + *A. versicolor* and applying irrigation during hot summer days.

Various studies on similar subjects of concern have suggested the implication of bioamendments and biocontrol agents to improve the efficacy of solarization alone against various diseases. Solarization integrated with crucifers effectively reduced corky root disease caused by *Phytophthora infestens* on tomatoes [38] and effectively controlled infestations of root-knot nematodes and weeds on melon and pepper [39]. The application of *T. harzianum* alone also controls various pathogenic fungal propagules in soil and withstands high temperatures of solarization; it gradually increases in the plant rhizosphere following the heat treatment [40]. In a current study, several biocontrol agents, including *Trichoderma harzianum*, *T. koningii*, *T. viride*, *Pseudomonas fluorescens*, *P. putida*, *A. niger*, *A. flavus*, *Penicillium citrinum*, *Serratia marcescens*, *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus amyloliquefaciens,* were tested in vitro against cumin wilt pathogens, and *Trichoderma* spp. was found the most capable with a 66 to 81% inhibition zone, followed by *Aspergillus* spp. and *Pseudomonas* spp. as effective bioagents with a 63–67% and 68 to 76% zone inhibition [41]. The integration of solarization with *T. harzianum* provided a significant control of Fusarium crown and root rot of tomato under field and greenhouse conditions [42]. Okon et al. [13] demonstrated the capacity of solarization and *T. harzianum* to induce resistance to foliar diseases in various plants systemically. This may be due to a direct or indirect effect on the plant by stimulating beneficial microorganisms in the rhizosphere [13]. Biocontrol agents also have an improved effect of solar heating on root-knot nematodes [43].

Observations with the survival of biocontrol agents in the present study suggested greater survival capabilities of *A. versicolor* than *T. harzianum.* The thermotolerant abilities of *A. versicolor* have been supported by earlier studies and have recorded its survival even at 60–62 °C [14,44]. *A. versicolor* produces heat shock proteins and thermotolerant enzymes, including Insulinase, with stability at 65 °C, which plays a crucial role in heat tolerance [45,46]. The application of bioamendments such as Brassica does not enhance the survival of *A. versicolor* in solarized soil but was found supportive in wet-only soil. In the arid region, combining *A. versicolor* with a food substrate as a soil amendment improved the survival of *A. versicolor* and pathogen control [47]. These studies demonstrate that a formulated

product improves the antagonistic effect of biocontrol agents mainly due to the availability of food and nutrients. In the present study, the greater survival of *A. versicolor* in wet solarized than nonsolarized plots can be attributed to its thermotolerant ability. In contrast, combined with Brassica, wet soil could have supplemented adequate nutrients to *A. versicolor*, resulting in a higher survival rate. Furthermore, the study clearly found a sensitivity of *T. harzianum* toward temperature. Kapulnik and Gamliel suggested a reduced cell membrane function beyond an upper-limit fluidity exceeded by high temperatures, concluding that mesophilic organisms are more sensitive to high temperature due to low-melting-point unsaturated lipids in cell membranes [48]. *T. harzianum* is a versatile biocontrol agent otherwise known to suppress soil-borne plant pathogens through parasitism, production of antagonistic chemicals, competition for the host and nutrients, and induction of resistance in plants against disease-causing pathogens [49,50]. In contrast, thermo-tolerant organisms survive soil solarization due to macromolecule stability at temperatures up to 60 °C. These observations collectively suggest biosolarization as a quick, economical, and long-term integrative approach for pathogen management, explicitly using Brassica amendment and biocontrol agents such as *T. harzianum* and *A. versicolor* in combination with soil solarization for control of cumin wilt-inducing pathogen. Integrative management strategies based on biosolarization or anaerobic soil disinfestations and applications of organic amendments are built on reliable historical research and the selection of different management strategies made in a manner that favors beneficial microorganisms, including biocontrol agents, to maximize their effect on phytopathogens. Disease-suppressive soils' physical, chemical, and biological characteristics strongly select different combinational management strategies [51]. More recently, the role of microbial communities in plant growth promotion, stress management, and disease control in plants has gained significant importance [52–65]. The specific metabolites in soil on biologically regulated disease control methods, such as biosolarization and anaerobic soil disinfestation, have gained interest, become the subject of research, and have revealed novel potential modes of disease control [52–59].

The study was conducted targeting Indian arid zones and, therefore, possibly more applications in similar arid lands with the same agro-climate. Environmental conditions in Indian arid zones have been reported to fit soil-solarization approaches [53]. Solarization approaches for control have trended in more than 50 countries, including hot and humid regions.

5. Conclusions

The study refers to developing system-based approaches for managing soil-borne plant pathogens. This study demonstrated the better management of *F. oxysporum* propagules in a hot-arid environment following the combined application of *A. versicolor*, soil-solarization, and Brassica amendments under a hot-arid climate. Suppression of wilt-inducing pathogenic propagules of *F. oxysporum* in soil could be achieved better by integrated management rather than individual control methods.

Abbreviations

Biocontrol agents: BCAs: *Fusarium oxysporum* f. sp. cumini: Foc; *Aspergillus versicolor*: *A. versicolor*; *Trichoderma harzianum*: *T. harzianum*; Brassica amendments: BA; Colony-forming units: CFUs; Analysis of variance: ANOVA; Wet soil: WS; Wet-solarized soil: W+SS; Dry soil: DS.

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