Journal of Scientific and Innovative Research 2021; 10(2): 34-42
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2021; 1(2): 34-42

Received: 17-06-2021
Accepted: 27-07-2021

Sunita Chandel
Department of Plant Pathology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan- 173230, HP, India

Savita Jandaik
Department of Plant Pathology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan- 173230, HP, India

Correspondence:
Sunita Chandel
Department of Plant Pathology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan- 173230, HP, India
Email: schandelmpp@yahoo.co.in

Non-chemical strategies in integrated management of wilt and stem rot diseases of chrysanthemum

Sunita Chandel, Savita Jandaik

Abstract

Wilt and stem rot caused by *Fusarium oxysporum* f sp. chyrsanthemi and *Rhizoctonia solani* are most devastating diseases, amounting to cause 18 -27 per cent loss under congenial environmental conditions in chrysanthemum. Due to many issues such as fungicide resistance, cost, and adverse effects, eco-friendly integrated approaches seem to be an applicable management method against the wilt and stem rot diseases of chrysanthemum. The objective of our present study was to evaluate the alternative methods such as soil amendments, biological agents and soil solarization (SS) alone and in integrated form which are cheap and easily adoptable having long-term benefits in combating the wilt and stem rot infection of chrysanthemum. Neem cake and cotton cake were found superior against Fusarium wilt pathogen with highest 68.11 per cent disease reduction while mustard cake showed highest effectiveness followed by neem and groundnut cakes to minimize the *Rhizoctonia solani* infection by 64.29 per cent. Fungal antagonists proved better in controlling disease compared to bacterial antagonist. For soil solarization, 25µ thickness polythene sheet resulted in less disease than 50 µ thickness. Integration of all the components resulted into excellent control of stem rot and wilt under field conditions due to additive effects. Maximum disease control (73.15 %) achieved in SS+ *T. harzianum* + Neem cake, SS+ *T. harzianum* + *T. viride*. A drastic decrease in the inoculums load of both pathogens recorded after two months period in solarized than unsolarized soils with varied microbial counts and almost negligible appearance of weeds in solarised plots.

Keywords: Soil solarisation, Biocontrol, Organic amendments, Integrated management.

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora* Tzvelev) belonging to the family Asteraceae is an economically important flower, native to the Northern Hemisphere, chiefly Europe and Asia, and is believed to have been originated in China [1]. China and India are the world's largest producers of chrysanthemums with an average of 56 billion stems produced from total cultivated area of 31,900 ha in Asia. In the global market, The Netherlands stands first in Chrysanthemum production followed by Germany and the UK [2].

Diseases are an unfortunate fact experienced during the growth period that reduces its cultivation greatly. Wilt (*Fusarium oxysporum* f. sp. *chrysanthemi*) and stem rot (*Rhizoctonia solani*) are widespread and destructive diseases causing huge losses, mainly in summer having a warmer climate [3,4]. The threat of the spread of these pathogens is of great concern because infected, asymptomatic vegetative cuttings may get distributed, which can disseminate the infection to new places. Continuous use of pesticides, fertilizers and the long period of cultivation in the same piece of land can create a monoculture environment. This aggravates the soil borne inoculum and had a negative impact on the soil health as well as on the plant health that ultimately led to stunting, withering leaves, few flowers per plant, and inferior flower quality [5]. Fusarium wilt and stem rot of chrysanthemum are difficult to manage because of the pathogen persistence in the soil and low availability of resistant varieties are known for its cultivation [6].

Various methods of cultural practices such as crop rotation, soil solarization, elevated beds, etc. are adopted in general for the control of the soil borne pathogens worldwide. Such strategies have an additive role in minimizing the *Fusarium* spp. and *Rhizoctonia* sp. populations. Beneficial microorganisms also play a vital role in soil ecosystems as they are warriors fighting against plant pathogens [7].

These microbes act both indirectly via competition for nutrients and space and directly by priming or activating the resistance without direct antagonistic effect towards the pathogens or through hyperparasitism or antibiosis [8-10]. Soil amendments of various kinds add organic matter with a high C:N ratio, the competition for nitrogen allows to decline the saprophytic activity of the *Rhizoctonia solani* and in turn its potential pathogenicity [11], enhance activity of soil microorganisms and release of the volatile chemicals that suffocate the pathogen structures. These mechanisms either kill the mycelium, spores, and resting structures of plant pathogens [12]. This study is a first attempt at using these strategies in managing soil borne diseases of chrysanthemum. The main goal of this approach was to evolve an effective, low cost, sustainable, environment friendly management strategy for producing disease-free preparative material and quality flowers.

MATERIALS AND METHODS

Isolation of Biocontrol agents: Composite soil samples were collected randomly from 8 to 10 cm depth of the rhizospheric soil layer from chrysanthemum nurseries and fields of research farms of Dr YS Parmar University of Horticulture and Forestry, Nauni, located in district Solan (LAT -309084º N, LNG-770999ºE) of Himachal Pradesh, India in clean dry sterile containers using sterile spatula. Soil samples were dried under laminar air flow and serial dilutions up to 10^{-6} were prepared. Test tubes containing soil dilution were shaken on vortex mixer (REMI, India) at 1500 rpm for 2 minutes in a centrifuge (Moxcare, India) and the aliquots(1mL) spread separately on to the 20ml Potato Dextrose Agar medium (Merck, India) for *Trichoderma* spp. isolation [13] and King's B consisting of ingredients K_2HPO_4-40g/lt; $MgSO_4$-04g/lt; protease peptone-200g/lt; glycerol-80ml/lt; agar-200 g/lt, (pH 70±02) for sub-culturing of bacterium (*Bacillus subtilis*) and plates were incubated at 25ºC in BOD incubators (Relitech, India) under white flourescent light conditions for 4-5 days. Bacterial antagonist was procured from the Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni and repeatedly sub-cultured on nutrient agar medium. The fungi were identified according to cultural and morphological characters described by Rifai [14-16].

Isolation and pathogenicity test of wilt and stem rot pathogens:

Isolation: Infected samples of chrysanthemum were collected from the roots and the collar stem portion from the same University Research Farms from where the biological control agents were isolated. After washing the tissues thoroughly in sterile water, the causal fungi are isolated from plant tissues exhibiting clear symptoms. The infected tissues along with small unaffected tissue were cut into small pieces (2–5 mm squares) and by using flame-sterilized forceps, they were transferred to sterile Petri dishes containing 01% mercuric chloride solution used for surface sterilization of plant tissues. The plant parts were transferred to PDA (Merck, India) plates and incubated in BOD incubator (Relitech, India) for 5-7 days for the complete growth of fungi. The resulted fungi were purified using the hyphal tips technique on Rose Bengal medium and then sub-cultured for future studies. The presence of chlamydospore, phialide and hypha were observed for *F. chrysanthemum* f.sp. *chrysanthemi* [17] under a light microscope (Olympus make, Japan) with magna pro software by using 40x magnification. Similarly, hyphal width, formation of septa, and the constriction and angle of branching were also observed in the case of R. solani has given in 'Biology and Pathology' by Parmeter [18] by taking minimum 20 observations.

Pathogencity tests: The pathogenicity of the wilt was assessed following a standard root dip method [19, 20] by preparing spore suspension. First step was to prepare the inoculum for which 15 ml of sterilized distilled water was poured on to the PDA plates containing fungal (*Fusarium oxysporum* f.sp. *chrysanthemi*) culture. From one week old fungal culture raised on PDA medium, mycelium and conidia were scratched off with the help of sterilized blade. The 15 ml of inoculum mixture (mycelia and conidia) was transferred into 500 ml conical flask and diluted by adding sterile distilled water (dH_2O) to make the final volume to 250 ml, the suspension was filtered with sterilized filter paper to remove the mycelial sheets and final concentration of 8 x 10^{-4} conidia/ml was adjusted with the aid of haemocytometer [21].

Preparation of the soil mixture:

The soil mixture was prepared by mixing top soil, well rotten farm yard manure (FYM) and sand in 3: 2:1 ratio, sterilized in autoclave at 121ºC for 15 minutes and cooled overnight, the sterilized soil was filled in the mercuric chloride (1%) surface sterilized plastic pots of 6 inches diameter (Dubey and Singh 2008). Roots of one-month-old cuttings of chrysanthemum procured from the nurseries farm were washed with tap water; air dried and wounded by pricking with the help of a sterile needle as well as clipped from the top upto 1.0 cm long [22]. The rooted cuttings after air drying were dipped in the above-prepared spore suspension in 500ml flasks kept in an orbital shaker at 100 rpm. Thereafter, fungus inoculated rooted cuttings were shifted to the plastic pots, three cuttings per pot with five replications [23]. The uninoculated roots which were treated with sterile distilled water served as control. The plants were kept for close observations for nearly 30 days in polyhouses having 12/12 hr of 35 ± 1ºC Day time and 26 ± 1ºC at nights with a humidity range of 76-78%. The plants were scored after 21 days based on a modified 0 to 4 scale as proposed by Schoonhoven and Pastor-Corrales [24].

Scores (0-4 scale)

1. Plants with 1-24% of leaf showing slight chlorosis.

2. Plants, abnormal growth with 25-49% of leaves showing chlorosis and/or slight curvature, folding effect & symptoms extending from lower to upper leaf

3. Plants, abnormal growth with 50 - 74% of leaves showing wilting, chlorosis, changing to brown colour and/or limited necrosis.

4. Plants are severely affected and show abnormal, stunted growth with ≥ 75% of the leaves showing wilt symptoms.

The disease severity index (DSI) was calculated according to the parameters in the disease scale [21]

$\Sigma (A \times n)/\Sigma (B)$

DSI = x 100(%)

A: disease scales; n: number of plants in specific scale; B: total number of plants

For establishing the Koch's postulates in the case of *Rhizoctonia solani*, seedlings assay test was performed on one-month established rooted cuttings of chrysanthemum, planted in a 6 inches diameter plastic pots containing autoclaved sterile soil. Pots were divided into two groups; fungus inoculated and non-inoculated. Each pot was filled with 2kg of autoclaved sterile soil containing three plants and replicated five times. For inoculated treatment, a week old of pure culture of *R.solani* prepared on PDA was reduced in fine pieces by scraping the upper mycelia and dissolving in 10 ml of distilled sterile water in test tubes to prepare inoculum suspension. Before 10 days of the actual planting time, soil was inoculated with inoculum suspension, 5 ml per pot. Plants were maintained under room temperature (25- 28 ºC) and watered to saturation after planting. After a month of planting, the seedlings were removed from the soil and their roots rinsed with tap water for critical examination of the symptoms of stem rot on the growing chrysanthemum plants. The plants were evaluated for stem rot infection on slightly modified scale [25] from 1 to 5, where 1 = no lesions, 2 = superficial water-soaked necrotic lesions, 3 = discrete, reddish or dark brown, deep necrotic lesions without stem girdling, 4 = extensive stem rot phase with stem girdling, and 5 = severely infected plants with drying effect and death.

Dual culture technique for screening of BCA's against test pathogens:

A dual culture technique [26] was followed to observe the antagonistic effect of BCA's by placing the 5mm of agar bit from the seven days pure colony of the BCA's (*Trichoderma* spp.) and pathogenic fungi were plated at equivalent from the periphery distance. Inoculated plates were

incubated in a BOD incubator at 25±°C under light conditions and percentage inhibition was calculated at 15 days post incubation by the formula: Inhibition (%) =R1-R2/R1x100 where, R1=linear growth of the pathogen in check plate; R2=linear growth of pathogen in *Trichoderma* inoculated plate. The experiment was performed by CRD and was replicated thrice.

Mass culturing of BCA's and test pathogen (s): According to Kaushal and Chandel [27] the mass culture of fungal biocontrol agents (BCA's) was prepared on a wheat bran: sand (3:1w/w) ratio by combining 2% sucrose and 5% dry soybean powder per kg. The prepared mixture (300g) was then placed in heat resistant polypropylene bags and sterilized in an autoclave at 15 lbs psi for 1 hour over two days. Sterilized bags were inoculated by adding 3-5 bits of 5mm size of the actively growing cultures of the fungal biological control agents and incubated at 24-25 °C for two weeks. Bacterium was mass multiplied in nutrient broth, incubated at 37 degrees celsius, and used in suspension form.

Organic amendments:

Five organic amendments, cotton seed cake, groundnut cake, neem cake, mustard cake, and saw dust, were purchased from the market and used in the current studies in the form of powder at a rate of 4g/kg of soil wt/wt.

***In vivo* evaluation of organic amendments and biocontrol agents for wilt and stem rot disease management**:

A field experiment was set under natural environmental conditions to check the effect of organic amendments and biocontrol agents to control the wilt and stem rot diseases of chrysanthemum. During the first week of May, 2015, one month old rooted cuttings of chrysanthemum cv. Purnima obtained from the Department of Floriculture and Architecture, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P) were planted in the field in randomized block design (RBD)-factorial. Various parameters of soil observed were sandy loam, slightly acidic having a pH range of 6.2 to 6.7 and soil temperature varied from 22-32 °C during the whole cropping season. Organic amendments in powdered form were mixed thoroughly in the soil@ 4 g wt/wt basis. Mass culture (200 g) of the fungal biological control agents prepared on wheat bran was used as a dry formulation containing about $20x10^4$ spore /ml in one gram of substrate at the time of application. Forty-eight hours old nutrient broth culture of bacterium antagonist containing $32x10^6$ cfu/ml was added in suspension form as soil drenched in each bed near the root zone of the chrysanthemum plants. The rooted cuttings were also subjected to dip treatment in biocontrol agents for 30 minutes prior to planting in the field. Both organic amendments and the biological control agents were added 15days prior to the addition of the test pathogens, *Fusarium oxysporum* f sp. *chrysanthemi* and *Rhizoctonia solani*. The pathogens were mass multiplied on maize grain medium [28] and incorporated into soil @ 100g/plot of 1x1 m^2 after 15 days of the establishment of the organic and BCAs in the soil. The rooted cuttings of chrysanthemum were planted and each treatment was replicated thrice. The data on disease incidence was recorded 30 and 60 days after the planting and calculated as: Disease Incidence (%) = Number of plants infected /Number of total plants observed.

Effect of soil solarization individually and in combination with organic amendments and biocontrol agents on wilt and stem rot diseases of chrysanthemum:

An experiment was carried out in the University's Research Farm to determine the effect of soil solarization practice using two, 25 and 50 (µ) thickness transparent ultraviolet-stabilized polythene sheets (Agriplast Tech, India Pvt. Lt) on wilt and stem rot disease progression, and in the second trial the role of most efficacious organic amendments and biological control.

All the plots were inoculated with the maize grain medium of the test fungi @ 100g/m^2 prior to 15 days of solarization and irrigated at regular intervals for establishment of the pathogens in the soil [29]. After 15 days of inoculation, soil samples (5g) were taken randomly from the unsolarized plots in triplicate each from two thickness sizes of the polythene sheet to enumerate the count of the pathogens before solarization under laboratory conditions by growing them on the selective medium. Soil was watered to field capacity and solarized plots were covered with polythene sheets for two months (March-April, 2016). The edges of polythene sheet were buried deep in the soil from all corners to make them air tight. On completion of the time period, the polythene sheet was removed and the soil samples were collected and brought to the laboratory to estimate the test fungi population. The *Fusarium* population was monitored periodically at 30 and 60 days using Komada's for *Fusarium oxysporum* f. sp. *chrysanthemi* [30] and *Rhizoctonia solani* on semi-selective medium [31], respectively and were identified as per the morphological characters listed in 'The Genus Fusarium' by Booth [32] and 'Biology and Pathology' by Parmeter [18]. Composite soil samples (5 g) were removed from the top 5-10 cm of each plot for dilution-plate analysis for pathogen count. One gram soil subsample from each plot was suspended in 99 ml of water and aliquot dilution samples 10^{-1} to 10^{-6} were prepared and 10^{-6} was pipetted into five Petri plates, possessing the constituents of specific media for both the test fungi. The medium was cooled to 45°C, and the plates were swirled before the medium solidified. All plates were incubated at 25°C temperature for 7 days before counting the colonies. This count was multiplied by the dilution factor which gave the number of colony-forming units per gram of soil. Whereas in the field after 8 weeks, all plants were rated as healthy or diseased with wilt or stem rot infection on the basis of visual symptoms in each category of solarized and unsolarized plots in different thicknesses of polythene sheets. The different kinds of weeds in numbers which appeared at various frequencies in the solarized and unsolarized plots were also recorded. Populations of microbial communities were enumerated in both the conditions by taking the soil samples in a similar way as described for the pathogen determination. These were multiplied on PDA (potato dextrose agar) under aseptic conditions. The colonies forming units (cfu) at respective dilutions of 10^{-3}, 10^{-5} and 10^{-6} for fungal, actinomycetes and bacteria were counted and multiplied by dilution factor.

The spore suspension of two best biological control agents, *Trichoderma viride* and *T. harzianum* and two most effective organic amendments, cotton seed cake and need cakes from initial screening were further tested individually and along with the solarization in order to see the integrated effect on the wilt and stem rot development in natural conditions alone as well as in combinations in 25 µ thickness ultraviolet stabilized polythene sheet which have shown good results compared to the 50 µ. The experiment was laid down in Randomized Plot Design with three replications. Each plot (35x35 cm) was planted with cv. Purnima rooted cuttings of chrysanthemum after soil solarization. Plots receiving integrated treatment were first mixed with 75 g $Ca(OH)_2$ per plot (about 2 g/ kg mix) before setting for soil sterilization for nitrogen need, and soil pH tested was around 6.4. Only N0 $_3$-nitrogen fertilization along with phosphorus and potash as per the recommended dosages were mixed well in these plots, as described by Woltz and Engelhard [33]. The soil temperature was noticed as 27° C in initial stages that later rose to around 48-50°C. Plots of 1x1m2 were developed, the soil was well pulverized, and all agronomical operations to raise the crop were followed on a regular basis. Diseased plants were observed regularly to record the disease status on the basis of symptomatological description. Plants were also examined for the vascular tissue for discoloration and evaluated as healthy or diseased as stated on the basis of visual symptoms and examination of the vascular tissue for discoloration.

Statistical Analysis

The experiments conducted in laboratory and field conditions were subjected to CRD and RBD, respectively and were analyzed using analysis of variance (ANOVA) followed by the Duncan Multiple Range Test ($p < 0.05$) in the Statistical Package for Social Sciences (SPSS) Software Version 24 (IBM, India).

RESULTS

Morphological based identification of the wilt and stem rot causing pathogens:

While examining the two-week-old culture of test pathogens grown on PDA, the culture of *F. oxysporum* f. sp. *chrysanthemi* appeared off white. The mycelium was hyaline, septate and branched, producing three kinds of spores viz. microconidia, often aseptate, hyaline whereas macroconidia were hyaline, but 7-9 septate and sickle shaped and the size of micro and macroconidia measured were 1.50 × 6.33 µm and 2.88 × 15.11 µm, respectively. Under *in vitro* conditions, chlamydospores were generally round and globose, and they formed individually as well as in a chain-like structure. The microconidia were hyaline, aseptate and sickle shaped. Macroconidia came in a variety of sizes and were found aseptate. Our microscopic examination results show that the fungus has typical mycelia that resemble *R. solani,* and that hyphal branching occurred at a right angle. The culture in the Petri plates were light brownish in colour and after 14-15 days formed small sized sclerotia, somewhat irregular shaped ranging from 1-2.8mm in diameter. The hyphal width almost ranged from 4.55 µm to 7.24 µm.

Pathogenicity test on chrysanthemum:

Pathogenicity test of the wilt pathogen (*Fusarium oxysporum* f.sp. *chrysanthemi*) revealed that isolated fungus could infect the roots of chrysanthemum (cv.Purnima) within 14-18 days, inducing typical symptoms of root rot and wilt with chlorosis of the lower leaves which later advanced to the upper portion of the plant resulting in partial and complete wilting of the plant when roots were given pin prick and root clip treatment together with dip in spore suspension showing 70 to 80% of disease severity index, maximum as per the scale. However, by plant growth test and soil infestation method, the stem rot causing fungus, *R. solani*, took nearly 18-21 days to successfully express the typical symptoms of the necrotic and girdling effect as formed in the natural field conditions. Lesions first appeared as water soaked, turned pale yellow, and later tan brown; ultimately leaf blight occurred on the stem. The stem showed necrotic superficial lesions, reddish or dark brown with stem girdling, lesions enlarged to form larger necrotic areas, turned brown and rotted. Later, the growing plants got dry and eventually dropped down.

Antagonist's identification:

The fungal antagonists (*Trichoderma harzianum*, *T viride*, *T hamatum* and *T virens*) predominant in the rhizospheric soil of chrysanthemum were identified as per the key description of their morphological characteristics. The colony varied from snow white to white, light green, green, and dark green with concentric rings, possessing water droplets in the culture, while the mycelia color was hyaline, phialides were flask shaped, arranged in divergent groups, single, lateral, pyramidal, and bearing conidia's were subglobose to globose, cell wall smooth.

Effect of organic amendments and biological control agents on disease development

The current findings regarding the effect of organic amendments (Table 1) recorded at 30- and 60-day time intervals reveal the highest (69.12%) wilt control in soils amended with neem cake after 30 days, followed by cotton seed cake (52.61%) over other treatments, whereas stem rot was checked up to the extent of 65.60% revealing the lowest disease status and highest percent disease controversies. The best treatments in order of effectiveness were cotton seed cake, neem cake and groundnut cake for reducing the wilt and stem rot diseases, respectively. However, neem cake and groundnut cake were recorded statistically at par in their efficacy to minimize the stem rot infection in chrysanthemum. After 30 days with less inoculum load, the incidence of both diseases was managed comparatively at a high level, but as the inoculums increased with the growing pathogens, the percent of disease control decreased beyond 60 days of observation.

In the *in vitro* dual culture experiment (Table 2), all four fungi and one bacterium cultures showed above 68 percent mycelium inhibition, with the highest reduction reaching 79.49% against both pathogens with antagonists, *Trichoderma harzianum* and *T. viride.* Individually, these resulted in 78.10 -79.03% and 79.47-80.87% mycelia inhibition of *Fusarium oxysporum* f. sp. *chrysanthemi* and *Rhizoctonia solani*, respectively. However, the bacterium was found to be the least effective compared to fungal antagonists.

The analysis of the data (Table 3) in relation to the individual testing of biocontrol agents revealed that two *Trichoderma* species, *T. harzianum* and *T. hamatum*, were statistically superior to other treatments by providing 75.37 and 72.42 per cent disease control in reducing *Fusarium* wilt infection, respectively, whereas *Trichoderma viride* provided the highest efficacy of about 75.47 per cent against stem rot.

Drastic reduction in the *Fusarium* wilt (*Fusarium oxysporum* f. sp. *chrysanthemi*) and stem rot (*Rhizoctonia solani*) infection with 25 µ and 50 µ thickness polythene sheets were observed (Table 4).

The wilt disease level (12.50%) was comparatively less in 25 µ which gradually reached to 21.25% in 25µ in solarized plots even after 60 days of observation. However, unsolarized plots recorded about 43.75% in 25µ which was less than 50 µ polythene sheet, accounting for 62.00 per cent of infection. The overall effect was quite less (23.13%) in solarized plots compared to 55.13% in untreated plots in both thicknesses, but the overall incidence of disease was reduced maximum (28.13%) in 25.

The stem rot of chrysanthemum was about 16.67% in 25 µ and 33.33% in 50 µ polythene sheet with an overall effect of 25% in solarized while 66.66% in unsolarized plots, which is quite high (Table 5).

Ten weeds were identified (Table 6), but only one weed species, *Medicago sativa* with a 10% population count was observed in solarized soil while all the species were enumerated in the untreated solarized soils. *Cynodon dactylon, Medicago sativa* were predominant with records of 59 and 58 per cent frequency of their occurrence. However, the least frequency occurrence (7%) was noted in the case of *Solanum nigrum* under unsolarized plots.

Before setting the experiment for solarization, the initial population count of the pathogens, *Fusarium oxysporum* f. sp. *chrysanthemi* and *Rhizoctonia solani* were 48.98 $x10^3$/g soil and 54.67 x 10^3/g soil, respectively, which were found to reduce drastically in solarized soils to 6.22 and 8.56 after a 60 days period of enumeration than 30 days (Table 7). The population of the pathogen, on the other hand, increased gradually in the two-month period and was recorded at 61.78 and 72.47 at a dilution factor of 10^{-3}.

When the microbial community such as fungi, bacteria, and actinomycetes in one gram of solarized soil were assessed, they resulted in an overall reduction from their initial count in the case of fungi, but a reverse trend was observed in actinomycetes and bacteria (Table 8).fungal count from 30 to 60 days was reduced to 38.64 c.f.u from 50.33 x 10^3, actinomycetes increased slightly from 29.24x10^5 to 30.36 c.f.u at 10^5 while bacteria increased maximum to 41.94 c.f.u from the initial count of 34.56 x 10^6. Individual effects of solarisation were also observed to have a general effect on fungal population, which decreased to a maximum of 15.92 c.f.u in solarized plots, whereas actinomycetes increased to 38.68 c.f.u and bacteria, showed a decreasing trend (19.66 c.f.u).

ii) Integrated effect on disease progress of wilt and stem rot: Soil solarized plots could reduce the effect of wilt to 68.50 and stem rot to 58.33 per cent when tested alone (Table 9).

However, wilt was reduced to a maximum of 70.0 percent in neem cake mixed plots, while disease was reduced to a maximum of 71.13 percent in *Trichoderma harzianum* amended soil mix along with soil solarization. In our studies, an additive effect was observed in two individual treatments, solarized and neem cake, as well as two combined treatments, SS+*T.h*+NC and SS+*T.h*+*T.v*, which reduced wilt to the highest level, 72.25 percent, with a disease range of 68-72.25%, and stem rot to 76.33 and 75.0 percent, respectively. It was further noticed that the next best treatments in order of efficacy were *Trichoderma harzianum* (*T.h*), SS+NC+CSC and SS+*T.v*+CSC towards stem rot by giving about 68.50 to 71.13 per cent reduction in disease level. Stem rot could be managed

better than wilt by giving comparatively more disease control of 65.66 per cent compared to 60.17 per cent.

Table 1: Effect of organic amendments on the wilt and stem rot of chrysanthemum

Organic amendments	Wilt Disease control (%) after days		Mean	Stem rot Disease control (%) after days		
	30	60		30	60	Mean
Cotton seed cake	54.88b	50.33b	52.61b	45.0c	29.0d	37.00c
Groundnut cake	33.28d	29.22d	31.25d	59.14b	52.86c	56.00b
Neem cake	71.66a	66.57a	69.12a	60.57b	55.57b	58.07b
Mustard cake	48.24c	41.36c	42.22c	66.85a	64.34a	65.60a
Saw dust	17.43e	10.10e	14.76e	25.12d	21.31e	23.22d
Mean	60.39	54.77	--	51.34	44.62	--

*The values shown with similar alphabets do not differ significantly from Duncan's Multiple Range Test ($p<0.05$)

Table 2: *In vitro* screening of antagonists (fungi, bacterium) against the causal organisms of wilt and stem rot diseases of chrysanthemum

Antagonists	Inhibition (%) (*Fusarium oxysporum* f. sp.*chrysanthemi*)	Inhibition (%) (*Rhizoctonia solani*)	Mean
Trichoderma viride	78.10a	80.87 a	79.49a
T. harzianum	79.03 a	79.47 b	79.25 a
T. hamatum	71.40 b	72.87 c	72.14b
T. virens	70.40 b	72.20 c	71.30b
Bacillus subtilis	68.47 c	70.33 d	69.40c

*The values shown with similar alphabets do not differ significantly from Duncan's Multiple Range Test ($p<0.05$)

Table 3: Effect of fungal and bacterial antagonists' application on reduction of wilt and stem rot of chrysanthemum

Antagonists	Disease control (%) after 60days		Mean
	Wilt	Stem rot	
Trichoderma viride	68.11c	75.47a	72.12a
T. harzianum	75.37a	69.16b	71.77a
T. hamatum	72.42b	65.88e	69.15b
T. virens	64.37d	67.55c	65.96c
Bacillus subtilis	55.18e	47.34d	51.33d
Mean	70.62	65.08	--

*The values shown with similar alphabets do not differ significantly from Duncan's Multiple Range Test ($p<0.05$)

Table 4: Effect of different thickness of polythene sheets on chrysanthemum wilt

Treatments	Per cent wilt incidence in thickness of polythene sheets (µ)		Mean
	25 µ	50 µ	
Solarized	12.50b	21.25b	23.13b
Unsolarized	43.75a	62.00a	53.13a
Mean	28.13	41.88	--

*The values shown with alphabets differ significantly from Duncan's Multiple Range Test ($p<0.05$)

Table 5: Effect of different thickness of polythene sheets on chrysanthemum stem rot

Treatments	Per cent stem rot incidence in thickness of polythene sheets (µ)		Mean
	25 µ	50 µ	
Solarized	16.67b	33.33b	25.00b
Unsolarized	58.33a	75.00a	66.66a
Mean	37.50	54.17	--

*The values shown with alphabets differ significantly from Duncan's Multiple Range Test ($p<0.05$)

Table 6: Effect of soil solarization on frequency of occurrence of weed population

Weeds	Weed Population in m^2 (%)	
	Solarised	Unsolarized
Artemisia sp.	-	31
Cynodon dactylon	-	58
Cyrus rotundus	-	10
Euphoria hirta	-	8
Medicago sativa	10	59
Parthenium sp.	-	18
Rumex sp.	-	10
Solanum nigrum	-	7
Sonchus sp.	-	24
Vicia sp.	-	18

- = Not found

Table 7: Effect of solarisation on the population of *Fusarium oxysporum* f. sp. *chrysanthemi* and *Rhizoctonia solani* in solarised and unsolarized beds

Days	Population (10^3 /g soil)					
	Fusarium oxysporum f. sp. *chrysanthemi*		Mean	*Rhizoctonia solani*		Mean
	Solarized beds	Unsolarized beds		Solarized beds	Unsolarized beds	
30	20.44a	56.66b	38.55a	10.44a	69.23b	39.84b
60	6.22b	61.78a	34.00b	8.56b	72.47a	40.40a
Mean	13.33	59.22	--	9.50	70.85	--

*The values shown with alphabets differ significantly from Duncan's Multiple Range Test ($p<0.05$)
Wilt pathogen: Initial count: 48.98 $x10^3$/g soil; Stem rot: Initial count: 54.67 x 10^3/g soil

Table 8: Effect of solarization on microbial population, fungi, bacteria and actinomycetes

Days	Population of fungal propagules ($x10^3$ c.f.u.g^{-1})		Mean	Population of actinomycetes propagules ($x10^5$ c.f.u.g^{-1})		Mean	Population of bacterial propagules ($x10^6$ c.f.u.g^{-1})		Mean
	Solarized beds	Unsolarized beds		Solarized beds	Unsolarized beds		Solarized beds	Unsolarized beds	
30	29.33a	55.69b	42.51a	33.22b	24.86a	32.04a	29.98a	56.98b	43.48a

60	15.92b	61.36a	38.64b	38.68a	20.04b	30.36b	19.66b	64.22a	41.94b
Mean	22.62	58.52	--	35.95	22.45	--	24.82	60.60	--

*The values shown with alphabets differ significantly from Duncan's Multiple Range Test ($p<0.05$)
Fungi: Initial count: 50.33 x 10^3 c.f.u/g soil
Actinomycetes: Initial count: 29.24 x 10^5 c.f.u/g soil
Bacteria: Initial count: 34.56 x 10^6 c.f.u/g soil

Table 9: Management of chrysanthemum wilt and stem rot using soil solarization, fungal antagonists and organic amendments

Treatments	Per cent disease control		Mean
	Wilt	Stem rot	
Soil solarization (SS)	68.50bc	58.33e	63.42d
Neem cake (NC)	70.00b	62.77e	66.38c
Cotton seed cake (CSC)	44.33h	46.67f	45.50h
Trichoderma harzianum (*T.h*)	66.67c	71.13b	68.90b
T.viride (*T.v*)	55.55f	64.23d	59.89f
SS+*T.h*+*T.v*	74.25a	75.00a	73.63a
SS+*T.h*+NC	51.81g	69.26b	60.54 de
SS+*T.h*+CSC	50.00g	60.00de	55.50g
SS+NC+CSC	54.25f	70.00b	62.10de
SS+*T.h*+NC	70.00b	76.33a	73.17a
SS+*T.v*+CSC	58.52d	68.50bc	63.51d
Mean	60.17	65.66	----

*The values shown with alphabets differ significantly from Duncan's Multiple Range Test ($p<0.05$)

DISCUSSION

The two test pathogens (*F. oxysporum* f. sp. *chrysanthemi* and *Rhizoctonia solani)* of chrysanthemum identified based on the basis of chlamydospore, micro and macro morphological characteristics and hyphal branching pattern using suitable media, slide cultures and the most updated keys for identification with sporulation on PDA plates. Similar results were reported by Ghosh et al. (1982) in the case of *Fusarium* spp. which possessed microconidia (1.5 – 4.2 × 6 – 15µ) with white colored septate mycelium, macroconidia (15.9-55 × 2.25-5.4 µ) and chlamydospores (7 to 12 µ). Sneh *et al*. [34] in their findings revealed the colony colour in *R. solani* ranged from light brown to dark brown and yellowish brown and hyphae bifurcated at the right angle and sclerotia were brown to dark in colour. The pathogenicity test elucidated the differences in the severity and morphology of the lesions caused by *Fusarium oxysporum* f. sp. *chrysanthemi* and *Rhizoctonia solani.* The pathogen, *F. oxysporum* f. sp. *chrysanthemi* could infect the roots of chrysanthemum (cv.Purnima) within 14-18 days and induce typical symptoms, while *R. solani* took nearly 18-21 days to express the typical symptoms as formed in the natural field conditions. In this study, a total of four fungal antagonists were identified as distinct species, *T. harzianum, T. viride, T. hamatum* and *T. virens* which were predominant in soils and were found morphologically similar to those reported by Gams and Bissett [15] and Castle *et al* [16].

The results of the organic amendments revealed that neem cake had the best control of wilt, followed by cotton seed cake, whereas in the case of stem rot infection, plots treated with mustard cake had the best disease control, followed by neem cake. Sharma et al [35] reported neem cake as the most effective treatment for reducing the incidence of carnation wilt by 16.67 per cent. Soil organic amendments also provide a diversified food base, which controls soil borne diseases and pests. These can diversify and change the microbial communities or population equilibrium in the soil and are made up of organic wastes, composts and peats [36].

Our *in vitro* results demonstrated that in a dual culture experiment, a clear zone by the antimicrobial activity of the *Trichoderma* antagonists developed which could be due to production of the antibiotics like chitinases, gluconases, trichodermol, trichodermini, peptiabols as reported by Harman [37] and Shoresh *et al* [38]. Fungal antagonists provided better than bacterial antagonists in inhibiting mycelial growth of the test pathogens. Chandel and Sharma [39] identified *T. viride* and *T. harzianum* as the most effective biocontrol agents when used as a root dip in combating stem rot infection in carnations. *Pseudomonas* spp. and *Bacillus* sp. were found efficacious in suppressing carnation wilt and improving plant growth. Soil solarization and amendments of *Trichoderma harzianum, T. viride*, neem cake and their combinations have been reported to be beneficial against Fusarium wilt of gladiolus. Rajendraprasad *et al* [40] reported the combined potential of *Trichoderma harzianum*-1 and *Pseudomonas fluorescence* effective in increasing germination and reducing pre- and post-emergence collar rot and damping-off of tomato in the pots when inoculated with *Sclerotium rolfsii*.

The experiment pertaining to individual testing of biocontrol agents disclosed that *T. harzianum*, *T. hamatum* and *T. viride were* more effective in reducing the Fusarium wilt infection than *Trichoderma viride* and *T.virens* whereas *Trichoderma viride* resulted in maximum disease control of 75.47 per cent against stem rot pathogen, *Rhizoctonia solani. Bacillus subtilis* was found to be the least effective against both pathogens under study. Ha [41] conducted experiments on several crops such as tomato, cucumber, peanut and durian and concluded that *Trichoderma* strains reduced the disease incidence caused by soil borne pathogens viz; *Rhizoctonia* spp., *Fusarium* spp., *Phytophthora* spp., *Sclerotium* spp. and *Pythium* spp.

Out of two thickness of polythene sheets tested for soil solarization studies, 25 µ proved comparatively more effective than 50 µ. In our studies, only one weed, namely *Medicago sativa*, was found in solarized plots. Kumar et al. 2015 reported plastic sheet mulch produces more yield of sunflower by conserving more moisture and having effective weed control. Abd-Elgawad *et al* [42] conducted soil solarization study for 3, 6, and 9 weeks and obtained a significant reduction in disease incidence and severity of black root rot of strawberries. Complete reduction in total count of all tested fungi i.e *Fusarium solani*, *Pythium* sp., and *Rhizoctonia solani* was obtained after 9 weeks. Marenco and Lustosa [43] concluded that solarization for nine weeks increased carrot yield and was effective for controlling more than half of the weed species recorded in carrot fields. The population of *Fusarium oxysporum* f. sp. *chrysanthemi* and *Rhizoctonia solani* was drastically reduced in solarized after 60 days of solarization in the current studies. Our studies are in agreement with Farrag and Fotouh [44] who reported that soil-solarization for eight weeks proved highly efficacious in reducing wilt and root-rot pathogens of cucumber plants.

In the present studies, microbial community such as fungi, bacteria and actinomycetes in one gram of solarized soil resulted in an overall reduction from their initial count in the case of fungi, but in actinomycetes and bacteria, a reverse trend was observed. The inference of the study was also supported by Sharma and Sharma [45] who reported the population of antagonistic microorganisms increased after solarization; however, a gradual decrease was experienced in total microbial population including fungi, bacteria and actinomycetes.

Integration of biocontrol agents, organic amendments and soil solarization had enhanced efficacy than the individual treatments in the management of the wilt and stem rot disease. Stem rot could be managed comparatively better than wilt using combinations of disease management approaches with higher disease control. A reduction in disease incidence due to the application of organic amendments with solarization has been reported in *Fusarium* spp. and *Phytophthora capsici* infestation in pepper [46, 47]. Soil solarization in combination with soil amendments and mycorrhizal fungi has also been reported to be effective in management of wilt disease of carnation [35].

CONCLUSION

The present study is the first attempt that signifies the integral approach in checking the most serious soil borne pathogens, *Fusarium oxysporum* f. sp. *chrysanthemi* and *Rhizoctonia solani*, responsible for inciting wilt and stem rot diseases in chrysanthemum. The most exciting feature of this research was to compose all the alternative eco-friendly components viz; soil solarization, soil amendment and biological control agents in integrated form after their individual testing in reducing the disease and weed levels, which has been authenticated to possess the potential to promote the antagonistic microbial colonies in the soil under field conditions. High temperature created with use of transparent thin polythene sheet (25µ) actually checked the pathogen's growth with activation of multiple resistance mechanisms induced by addition of antagonist's and organic amendments. Therefore, use of solarization, neem cake practices alone and the integral form (SS+*Th*+NC and SS+*Th*+*Tv*) is advisable with these and similar soil borne diseases to mitigate the huge losses in commercial ornamental flowers.

Acknowledgements

We would like to appreciate and thanks the University Grant Commission, New Delhi for providing funds in the form of the research project to carry out the present investigation

Conflict of Interest

None declared.

REFERENCES

1. Bose TK, Yadav LP, Pal P. Chrysanthemum Commercial Floriculture. 2nd Kolkata, Naya Prokash 2002: 463-602.
2. Singh PK, Kumar V. Fusarium wilt of chrysanthemum-Problems and prospects. Plant Pathology and Quarantine.2014; 4(1): 33-44.
3. Engelhard AW, Woltz SS. Pathogenecity and dissemination of wilt pathogens of chrysanthemum (Abstract). Phytopathology 1973;63:441.
4. Fisher NL, Toussoun TA. Symptomatology and colonization of chrysanthemum infected with Fusarium oxysporum f sp. chrysanthemi (Abstr). Phytopathology.1981;71: 1043-1050.
5. Duineveld BM, Kowalchuk GA, Keijzer A, van Elsas JD, van Veen JA. Analysis of bacterial communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-Amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. Applied Environmental Microbiology. 2001; 67:172–178.
6. Garibaldi A, Bertetti D, Gullino ML.Susceptibility of chrysanthemum and paris daisy varieties to several isolates of Fusarium oxysporum f sp. chrysanthemi Commum .Agriculture Biolological Science 2009;74(3):651–657.
7. Bai L Cui, JQ Jie, WG, Cal BY. Analysis of the community compositions of rhizosphere fungi in soybeans continuous cropping fields. Microbiological Research. 2015;180:49-56.
8. Conrath, U, Beckers GJM, Langenbach JG, Jaskiewicz MR. Priming for enhanced defense. Annual Review of Phytopathology. 2015;53: 97–119.
9. Spadaro D, Droby S. Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. Trends in Food Science Technology.2016; 47:39–49.
10. Raaijmakers JM, Mazzola M. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Annual Review of Phytopathology. 2012;50:403–424.
11. Papavizas GC. Colonization and growth of Rhizoctonia solani in soil. In: Parmeter Jr ed Rhizoctonia solani Biology and Pathology, University of California Press, Berkley 1970, 255.
12. Ghorbanpan A, Salimi A, Ali M, Ghanbary T, Pirdashti H, Desetani A. The effect of Trichoderma harzianum in mitigating low temperature stress in tomato (Solanum lycopersicum) plants. Sciencia Horticulturae. 2018;230 :134-141.
13. Rodriguez-kabana R. An improved method for assessing soil-fungus population density. Plant Soil.1967; 26(2): 393-396
14. Rifai MA. A revision of the genus Trichoderma Mycological Papers,1969 Common wealth Mycological Institute, Kew, Surrey, England 1969,116.
15. Gams W, Bissett J. Morphology and Identification of Trichoderma. In: Trichoderma and Gliocladium: Basic Biology, Taxonomy and Genetics, Harman, G.E. and C.P. Kubicek (Eds.). Vol. 1, Taylor and Francis, London, UK 1998, 3-34.
16. Castle A, Speranzini D, Rghei G, Alm, Rinker D, Bissett J. Morphological and molecular identification of Trichoderma isolates on north American mushroom farms. Applied Environmental Microbiology. 1998; 64(1): 133-137.
17. Leslie JF, Summerell BA, Bullock S. The Fusarium laboratory manual, 1st edition Wiley Blackwell. 2006.
18. Parmeter JR. Rhizoctonia solani, Biology and Pathology University of California Press, London. 1970; 255.
19. Herman R, Perl-Treves R.Characterization and inheritance of a new source of resistance to Fusarium oxysporum f sp. melonis Race 12 in Cucumis melo. Plant Disease 2007; 91(9): 1180-1186.

20. Karimi R, Owuoche JO, Silim SN.Inheritance of Fusarium wilt resistance in pigeonpea [Cajanus cajan (L) Millspaugh. Indian J Genet Plant Breed. 2010;70(3): 271-276
21. Mwaniki PK, Abang MM, Wagara IN, Wolukau JN, Schroers HJ. Morphology, pathogenicity and molecular identification of Fusarium spp. from wilting eggplants in Tanzania. African Crop Sciences Conference Proceedings.2011; 10: 217-221.
22. Cumagun CJR, Aguirre JA, Relevante CA, Balatero CH. Pathogenicity and aggressiveness of Fusarium oxysporum Schl in bottle gourd and bitter gourd. Plant Protection Science. 2010; 462:51-58.
23. Vakalounakis DJ, Wang Z, Fragkiadakis GA, Skaracis GN, Li DB. Characterization of Fusarium oxysporum isolates obtained from cucumber in China by pathogenicity, VCG, and RAPD. Plant Disease.2004; 886:645-649.
24. Schoonhoven A, Pastor-Corrales MA. Standard system for the evaluation of bean germplasm CIAT, California, Columbia 1994, 20.
25. Muyolo NG, Lipps PE, Schmitthenner AF. Reactions of dry bean, lima bean, and soybean cultivars to Rhizoctonia root and hypocotyls rot and web blight. Plant Disease. 1993;77:234-238.
26. Skidmore AM, Dickinson CH. Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. Transaction of British Mycology Society. 1976: 57-64.
27. Kaushal S, Chandel S. Enhancing the shelf life of Trichoderma species by adding antioxidants producing crops to various substrates. Journal of Crop Protection.2017; 6(3): 307-314.
28. Dohroo NP, Sharma SL. Biological control of rhizome rot of ginger in storage with Trichoderma viride. Indian Journal of Plant Pathology. 1984; 2:185-186.
29. Martyn RD, Hartz TK.Use of soil solarisation to control Fusarium wilt of watermelon. Plant Disease.1986;70:726-766.
30. Komada H. Development of a selective medium for quantitative isolation of Fusarium oxysporum from natural soil. Review of Plant Protection Research. 1975; 8:114-125.
31. Gutierrez WA, Shew HD, Melton TA. A semi-selective medium to isolate Rhizoctonia solani from soil and tissue. Plant Pathological Extension.2001;1-2.
32. Booth C. The genus Fusarium McGraw and Hill company, London. 1971; 273.
33. Woltz SS, and Engelhard AW. Fusarium wilt of chrysanthemum: Effect of nitrogen source of and lime on disease development. Phytopathology. 1973;63: 155-15.
34. Sneh B, Burpee L, Ogoshi A. Identification of Rhizoctonia species. Ann Phytopathological Society Press, St Paul, Minnesota 1991,133.
35. Sharma S, Raj H, Sharma N. Integration of Soil solarization, Arbuscular Mycorrhizal Fungi, Trichoderma viride, Azotobacter chroococcum and soil amendments for the management of carnation (Dianthus caryophyllus L) Wilt (Fusarium oxysporum f.sp. dianthi (Prill and Del) Snyd and Hans). International Journal of Current Sceince and Microbiological and Applied Sciences. 2019; 8 (1):2484-44.
36. Nakamura K, Watanabe S, Ozaki H, Ikeura Y, Kotani A. Soil temperature and moisture environments: Lot-management water requirements associated with soil solarisation. Farm Agrico. 2011;631:2-10.
37. Harman, GE. Overview of mechanisms and uses of Trichoderma spp. Phytopathology. 2006; 96(2): 190-194.
38. Shoresh M, Mastouri F, Harman G. Induced systemic resistance and plant responses to fungal bio-control agents. Annual Review of Phytopatholgy.2010; 48: 21-43.
39. Chandel S, Sharma S. Botanicals, biofumigants and antagonists application in managing stem rot disease caused by Rhizoctonia solani Kuhn in carnation. Journal of Biopesticides. 2014;7(1): 3-10.
40. Rajendraprasad M, Sagar B, Vidya D, Uma G, Rao SR, Loteshwar. Biocontrol of tomato damping off caused by Sclerotium rolfsii. Journal of Entomology Zoology Studies 2017;5(5):113-119.
41. Ha TN. Using Trichoderma species for biological control of plant pathogens in Veitnam. Journal of the International Society for Southeast Asian Agricultural Sciences. 2010;16(1): 17-21.
42. Abd-Elgawad, MMM Elshahawy IE, Abd-El-Kareem F. Efficacy of soil solarization on black root rot disease and speculation on its leverage on nematodes and weeds of strawberry in Egypt. National Research Centre. 2019; 43: 175.
43. Marenco RA, Lustosa DC. Soil solarization for weed control in carrot. Pesquisa Agropecuária Brasileira.2000;35(10): 2025-2032.
44. Farrag ESH, Fotouh YO. Solarization as a method for producing fungal-free container soil and controlling wilt and root-rot diseases on cucumber plants under greenhouse conditions. Arch Phytopathology Plant Protection.2010;43(6): 519-526.
45. Sharma M, Sharma SK. Effect of soil solarization on soil microflora with special reference to Dematophora necatrix in apple nurseries Indian Phytopathology.2002;55(2): 158-162.
46. Martinez MA, Martinez MC, Bielza P, Tello J, Lacasa A. Effect of biofumigation with manure amendments and repeated biosolarization on Fusarium densities in pepper crops. Journal of Indian Microbiology and Biotechnology.2011;38:3–11.
47. Nunez-Zofio M, Larregla S, Garbisu C.Application of organic amendments followed by soil plastic mulching reduces the incidence of Phytophthora capsici in pepper crops under temperate climate. Crop Protection. 2011; 30:1563–1572.