Plant Archives Vol. 25, No. 1, 2025 pp. 2813-2822 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.1.407

COMPARATIVE STATUS OF FOLIAR DISEASES AND SOIL MICROBIOME IN NATURAL AND CHEMICAL FARMING SYSTEMS OF APPLE PRODUCTION

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(Date of Receiving-25-12-2024; Date of Acceptance-16-03-2025)

ABSTRACT

Apple production can be managed using natural or chemical farming practices. Each system impacts the incidence of foliar diseases and the composition of the soil microbiome differently. With the aim to compare these impacts, a study was conducted during 2021-2022 on ten apple orchards in three different blocks of Shimla district of Himachal Pradesh. Overall, incidence and severity of Marssonina blotch (42.85%, 32.32%) and Alternaria leaf spot (28.84%, 16.42%) of apple was reported to be higher in Chemical Farming System (CFS) as compared to Natural Farming System (NFS) (34.63%, 22.87% and 22.24%, 11.91%) of apple production. Fungal and bacterial count were recorded to be higher in NFS (34.45 and 33.45 cfu g^{-1} of soil) as compared to CFS (26.80 and 24.05 cfu g^{-1} of soil). This comparative analysis indicates that NFS generally foster a healthier and more diverse soil microbiome compared to CFS. This has significant implications for soil health, nutrient cycling, disease suppression, and overall sustainability of apple production.

***Key words*:** Alternaria leaf spot, fungal and bacterial count, Marssonina blotch, rhizosphere.

Introduction

Apple (*Malus* × *domestica* Borkh.) is a deciduous plant of the Rosaceae family and is most important fruit crop of temperate region. The plant is cultivated as a fruit tree worldwide in New Zealand, Asia, USA, Europe and southern parts of America and Africa. In India, apple is produced predominantly in north-western Himalayan states like Jammu and Kashmir, Himachal Pradesh and Uttarakhand contributing 90 per cent of the total production in the country (Wani and Songara, 2017). In addition, its cultivation has been extended to Arunachal Pradesh, Sikkim, Nagaland, Meghalaya and Nilgiri hills of Tamil Nadu, where mild temperate climatic conditions prevail. In Himachal Pradesh, apple is grown in Shimla, Kullu, Kinnaur, Mandi, Chamba and Sirmaur districts (Chand *et al*., 2017). These hilly areas of Himachal Pradesh are blessed with high agro-climatic suitability for high-value fruit crop production, also popularizing it by the name “Fruit Bowl of the Nation” (APEDA, 2006).

Apples are one of the most widely consumed fruits due to their wide- range of beneficial effects on human health. A high intake of apples can prevent chronic diseases and reduce the risk of lung cancer, asthma, type-2 diabetes, thrombotic stroke, and ischemic heart disease (Hansen *et al*., 2009; Chai *et al*., 2011). These benefits are associated with large content of structural cell walls and polysaccharides (Sun-Waterhouse *et al*., 2008) as well as various phytochemical antioxidants present in apple (Devic *et al*., 2010).

Area under apple cultivation in India has increased tremendously from 241.88 thousand hectares (2001-02) to 3.13 lakh hectares with production of 24.37 lakh metric tonnes (NHB, 2021). Total area under fruit production in Himachal Pradesh is 6.96 lakh hectares while apple is grown in an area of 1.15 lakh hectare with a production of 6.44 lakh metric tonnes (NHB, 2021). Though the area and production of apple crops in Himachal Pradesh have increased over the past few decades, productivity per

unit area has not increased as much. This is because of the various diseases affecting the fruit quality and yield. These diseases include Apple scab (*Venturia inaequalis*), Alternaria leaf spot (*Alternaria mali*), Marssonina leaf blotch (*Marssonina coronaria*), Powdery mildew (*Podosphaera leucotricha*), White root rot (*Dematophora necatrix*) and Collar rot (*Phytophthora cactorum*).

From past years, farmers are using fungicides and pesticides to prevent various diseases and pests, the use of which ultimately leads to many side effects on environment as well as on human health. As human have now become more health conscious, demand for chemical-free food is increasing (Pillai *et al*., 2021). Farmers are now shifting to non-chemical methods of disease management among which natural farming is one. 'Natural farming' means farming with nature and without chemicals. In this chemical free approach, chemicals are replaced with biological pesticides like cow dung, cow urine, jaggery and pulse flour for crop protection. This new emerging method of farming has many benefits like improvement in soil fertility, yield and quality of the produce, besides protecting from the side effects of chemical methods such as magnification, pollution, and carcinogenic elements (Bishnoi and Bhati, 2017) in comparison to chemical methods (where farmers use different chemicals as fertilizers and fungicides).

Microbes are a natural component of the agricultural environment. They interact with crop plants, support ecological balance, and contribute significantly to biodiversity in agro- ecosystems. Crop plants rhizosphere, endosphere and phyllosphere are important microenvironments and homes for microorganisms. Microbiomes include antagonists, pathogens and mutualistic symbionts that may be advantageous or harmful to crop plants, respectively (Granado *et al*., 2008). It has been observed that various farming methods and farm management techniques have an impact on the microbial populations in crop plants soil- root environments (Gattinger *et al*., 2007).

The soil microbiome plays a crucial role in the health and productivity of agricultural systems, including apple orchards. Understanding the differences between natural and chemical (conventional) farming systems is essential for making informed decisions about agricultural practices. Below is a comparative analysis of foliar diseases and soil microbiome status in natural and chemical farming systems for apple production in Shimla district of Himachal Pradesh.

Table 1: Description about study orchard sites.

Sites	Village/ Block	AT	Em	GPS Co-ordinates
Site 1	Lafughati (Theog)	LG	2300	*NFS - 31°R"10'21.3"N 77°R"22'38.5"E
				**CFS - 31°R"10'49.5"N 77°R"22'31.3"E
Site 2	Sariuoon (Theog)	SR	2203	NFS - 31°R"7'38.7"N 77°R"23'4.8"E
				CFS - 31°R"7'48.6"N 77°R"23'14.2"E
Site 3	Himari (Theog)	HM	2218	NFS - 31°R"7'59.5"N 77°R"23'24.7"E
				CFS - 31°R"7'52.9"N 77°R"24'8.9"E
Site 4	Mandhol (Jubbal)	MD	1083	NFS - 31°R"7'50.5"N 77°R"42'48.5"E
				CFS - 31°R"7'51.3"N 77°R"42'49.2"E
Site 5	Sainji (Rohru)	SJ	1876	NFS - 31°R"12'20.6"N 77°R"47'24.9"E
				CFS - 31°R"12'10.8"N 77°R"47'14.3"E

AT: Abbreviated Term; **Em:** Elevation (m amsl); **NFS- Natural Farming System; **CFS- Chemical Farming System*

Materials and Methods

Orchard Sites and Management Practices

The present study was focused on commercial apple orchards in three different blocks (Theog, Jubbal and Rohru) of Shimla district of Himachal Pradesh. The experiment took place in adjacent commercial, irrigated apple orchards, one natural and one conventional, each approximately 1.0 ha in size, to avoid any pedoclimatic impact. In total, five orchards under natural farming system (NFS) and five orchards under chemical farming system (CFS) of apple production were selected for comparison. The variety used was "Starking Delicious" grafted onto seedling rootstock. All the sampled trees were of uniform age and size. Detailed description of study orchard sites has been mentioned in Table 1. Also, the practices followed by the farmers of study orchard sites in both the farming systems has been mentioned in Table 2.

Survey

A periodic survey of selected orchards was conducted during Feb-July in the year 2021 and 2022 to study comparative severity of two major foliar diseases of apple *i.e.*, Marssonina leaf blotch (*Marssonina coronaria*) and Alternaria leaf spot (*Alternaria mali*).

To record disease severity, 40 plants were selected

randomly in each orchard. Disease severity was recorded by giving score to the affected area of leaf observed. For every disease, a particular scale was followed to record the disease severity.

Disease severity scale for Marssonina leaf blotch (*Marssonina coronaria*): 0-4 scale (James, 1974)

Grade	Per cent disease on leaves	Description of symptoms
0	0.0	Leaves completely healthy with no blotch symptoms
1	0.1-25.0	Leaves show light infection, disease mainly on the lower portion of the plant
2	25.1-50.0	Up to 50 per cent portion of the leaves infected
3	50.1-75.0	About 75 per cent portion of the leaves infected and the leaves appear to be blotched
4	75.1-100.0	Almost the whole of the leaves infected

Disease severity scale for Alternaria leaf spot of apple (*Alternaria mali*): 0-5 scale (Horsfall and Barrat, 1945)

Score	Severity
0	No symptoms
1	1-3 per cent of leaf area covered with lesions
2	4-6 per cent of leaf area covered with lesions
3	7-12 per cent of leaf area covered with lesions
4	13-25 per cent of leaf area covered with lesions
5	26-50 per cent of leaf area covered with lesions

The per cent disease severity was calculated by the formula given by McKinney (1923)

$$\text{Disease severity (\%)} = \frac{\text{Sum of all disease rating}}{\text{Total no. of ratings} \times \text{Maximum grade}} \times 100$$

Soil Sampling and microbial analysis

From the selected orchards, soil samples were collected in the month of October to determine the fungal and bacterial count. From the soil samples collected from each orchard, microbial count of fungi, bacteria were determined by using Serial Dilution Method (Aguilera *et al*., 1999). One gram soil from the composite sample was taken in 100 ml sterilized distilled water in 250 ml flask, shaken well on orbital shaker. Dilutions were made from 1×10^{-1} to 1×10^{-6} and growth was taken on specific media. Potato Dextrose Agar medium was used for the isolation of fungi and Nutrient Agar medium was used for the isolation of bacteria.

For fungal count, growth was taken at 1×10^{-1} and 1×10^{-2} dilutions whereas for bacterial count, growth was taken at 1×10^{-3} and 1×10^{-4} dilutions. Spread plate method was followed to get the growth of fungi and bacteria. 0.1 ml of each dilution was spread on Petri plates containing solidified media and were incubated at 25±1R°C. Each dilution was replicated thrice. After every 24 hrs, observation was taken for the appearance of colonies. To count the number of colonies, viable count technique was used. The colony forming unit (CFU) per volume of aliquot plated was calculated by the formula:

$$\text{Cfu g}^{-1}\text{ of soil} = \frac{\text{No. of colonies obtained} \times \text{Reciprocal of dilution used}}{\text{Volume of sample used}}$$

Purification and identification of isolated microflora

Different fungal colonies appeared on PDA medium were picked up, purified by hyphal tip method, and maintained on PDA slants. Bacterial colonies were also purified by streaking and were maintained on NA slants. Fungal microflora was identified based on morphological and cultural characteristics. For bacterial microflora colony morphological characteristics like colour and appearance of the colony and biochemical characteristics were studied. The bacterial antagonists were sent to CSIR-NCIM Pune, Maharashtra and Biokart Genomic Laboratory, Bengaluru, Karnataka for their identification.

Different biochemical tests performed to study biochemical characteristics are as follows:

Gram Staining

Gram staining was done to classify bacterial species into two major groups: gram- positive bacteria and gram-negative bacteria. In this process, heat fixed smear of bacteria were made and smear was covered with crystal violet for 30 seconds. Slide was washed with distilled water for a few seconds using wash bottle. Again, smear was covered with grams iodine solution for 60 seconds and further washed with ethyl alcohol. Then slides were washed with distilled water and drained. Safranin was applied to smears for 30 seconds (counter-staining) and finally washed with distilled water and blot dried with absorbent paper. Slides were examined microscopically (Aneja, 2003).

Starch Hydrolysis or Amylase Production Test

Starch agar medium was used for this test. Single streak inoculation of each bacterium was made in center of each labelled plate having starch agar media. Inoculated plates were kept in incubator and were observed for 48 hrs for their growth. Surface of the plates were flooded with the iodine solution for 30 seconds. Plates were observed for starch hydrolysis around the line of the growth of the bacteria.

Table 2: Comparative information about the practices followed by the farmers of study orchard sites.

Practices	Natural Farming System	Chemical Farming System
Mulching	Straw mulch	Straw mulch
Green manuring	Pea, rajmah, mustard, sunflower, French bean, coriander, potato, maize	nil
Farm yard manure	Applied in the form of ghanjee vamrit (4 kg plant^{-1})	5-10 kg plant^{-1}
Chemical fertilizer	nil	Urea- 1 kg plant^{-1}Calcium nitrate- 1 kg plant^{-1} MOP- 1 kg plant^{-1}12:32:16 or 15:15:15 – 1 kg plant^{-1} SSP – 300 g plant^{-1}
Jeevamrit	Once in a month as 10% foliar spray and drenching @ 8-10 L plant^{-1}	nil
Weed control	Manually	Manually
Pests and diseases control	• Buttermilk- against foliar diseases • Stem paste (turmeric, linseed oil, cow urine, cow dung, soil, garlic, green chilli, walnut leaves, and asafoetida)– against soil borne diseases • Bhramastra, Agniastra, Ash, Sonthastra- against insect-pests (On the onset of diseases/ insect-pests at an interval of 10-15 days)	captan (600 g 200 L^{-1}) at green tip stage mancozeb (600 g 200 L^{-1}) at walnut stage carbendazim (100 g 200 L^{-1}) at pink bud stage tebuconazole (126 ml 200 L^{-1}) at walnut stage or pre harvesthexaconazole (100 ml 200 L^{-1}) petal fall fenazaquin (50 ml 200 L^{-1})- against mites at fruit developmentmalathion (200 ml 200 L^{-1}) - at pre harvest stage for aphidschloropyriphos (400 ml 200 L^{-1}) – against woolly apple aphid
**The composition of Ghanjeevamrit, Jeevamrit, Bhramastra, Agniastra and Sonthastra has been given in Appendix-I.*		

Indole Test

Peptone broth (15g peptone in 1lt of distilled water) was prepared and bacteria were inoculated. Growth was observed for 24-48 hrs. 0.5 ml Kovac's reagent was added to each test tube and observation was taken. Colour change of the reagent was observed.

Citrate Utilization Test

Simmons citrate agar medium was poured in the Petri plates and plates were cultured with bacteria. These were allowed to grow for 24-48 hours and then observation was taken to see the change in colour. Colour change was positive indication for citrate test (Aneja, 2003).

Oxidase Test

For oxidase test, oxidase discs were used. Oxidase discs were first wetted with distilled water and loopful culture of bacteria were transferred to the discs and observed for two-three minutes.

Triple Sugar Iron (TSI) Test

For this test, Triple Sugar Iron Agar was used.

Catalase Test

For this test, hydrogen peroxide was used. A drop of 3 per cent of hydrogen peroxide was taken on slide and bacteria was spread on it. Observation was recorded for the appearance of bubbles.

Statistical Analysis

The data recorded was analyzed by using MS-Excel and OPSTAT as per the design of the experiment (Gomez and Gomez, 1984).

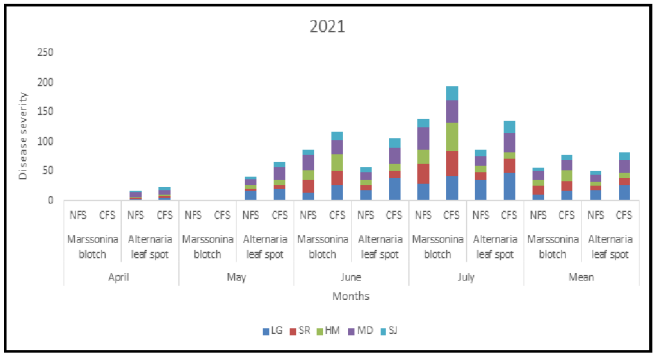

Fig. 1: Comparative severity of Marssonina blotch and Alternaria leaf spot under natural (NFS) and chemical farming system (CFS) of apple production during 2021.

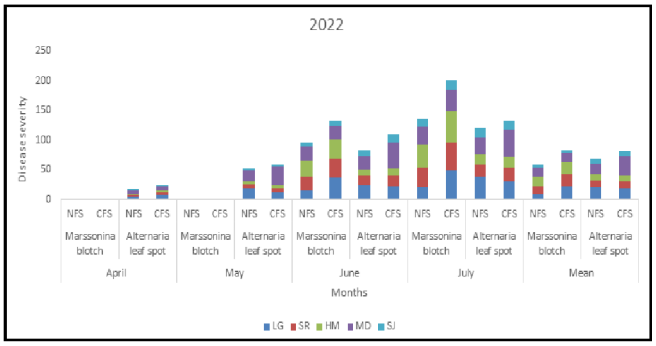

Fig. 2: Comparative severity of Marssonina blotch and Alternaria leaf spot under natural (NFS) and chemical farming system (CFS) of apple production during 2022.

Table 3: Comparative status of fungal microflora of soils under natural and chemical farming systems of apple production.

Fungal Count (cfu g^{-1} of soil)							
Location	NFS			CFS			Per cent increase over CFS
	Dilutions			Dilutions			
	10^3	10^4	Mean	10^3	10^4	Mean	
LG	19.50 (4.29)	9.50 (4.97)	**14.50 (4.63)**	11.50 (4.06)	6.00 (4.77)	**8.75 (4.42)**	39.66 (4.64)
SR	66.00 (4.82)	32.50 (5.51)	**49.25 (5.17)**	40.00 (4.60)	21.50 (5.33)	**30.75 (4.97)**	37.56 (3.87)
HM	38.50 (4.59)	26.00 (5.41)	**32.25 (5.00)**	30.50 (4.48)	25.00 (5.40)	**27.75 (4.94)**	13.95 (1.20)
MD	44.50 (4.64)	28.00 (5.44)	**36.25 (5.04)**	35.00 (4.54)	22.50 (5.35)	**28.75 (4.95)**	20.69 (1.88)
SJ	46.00 (4.66)	34.00 (5.50)	**40.00 (5.10)**	44.50 (4.68)	31.50 (5.50)	**38.00 (5.07)**	5.00 (0.49)
Mean	**42.90 (4.60)**	**26.00 (5.31)**	**34.45 (4.99)**	**32.90 (4.47)**	**23.30 (5.27)**	**26.80 (4.87)**	22.21 (2.39)

Figures in parentheses are log transformed values

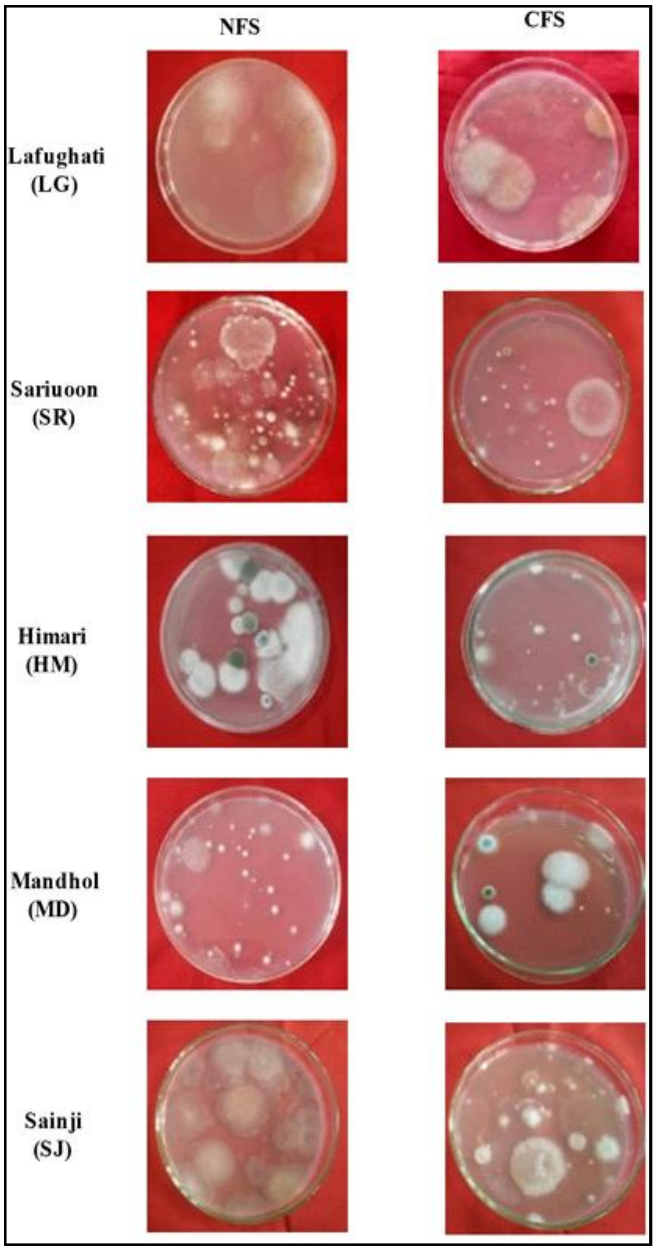

Fig. 3: Fungal microflora isolated from soils of natural and chemical farming systems of apple production (NFS- Natural Farming System, CFS- Chemical Farming System).

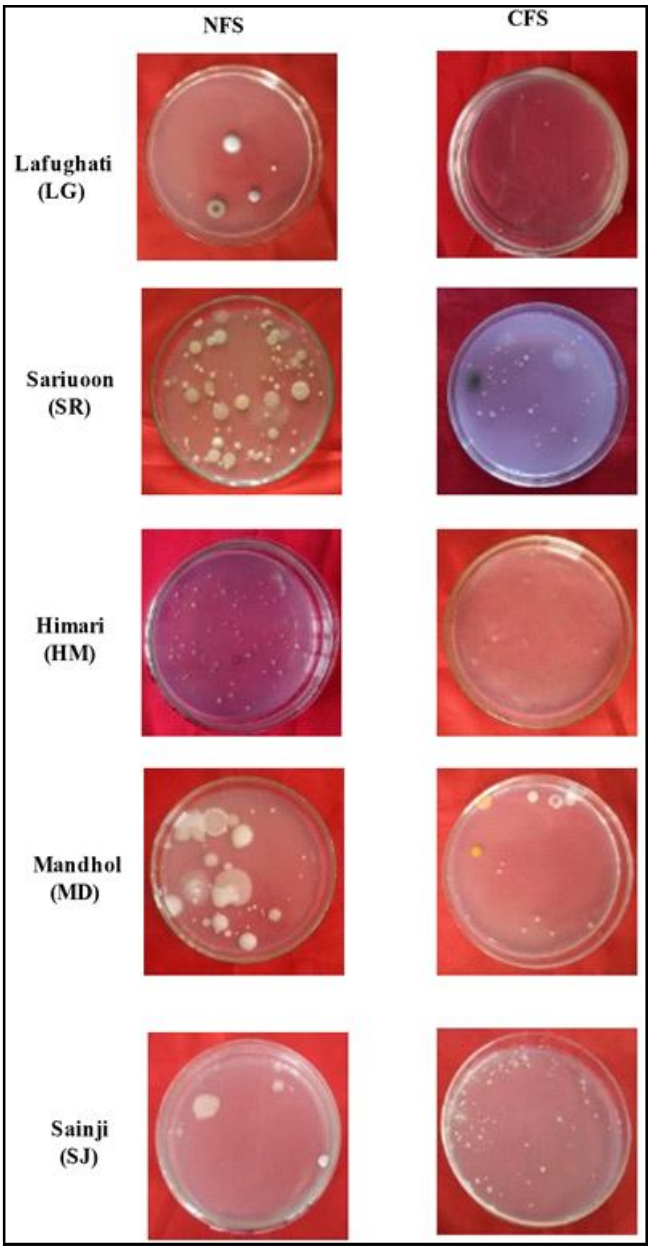

Fig. 4: Bacterial microflora isolated from soils of natural and chemical farming systems of apple production (NFS- Natural Farming System, CFS- Chemical Farming System).

Results and Discussions

Comparative status of foliar diseases in NFS and CFS of apple production

The data regarding comparative severity of Marssonina blotch and Alternaria leaf spot under natural (NFS) and chemical farming system (CFS) of apple production during 2021 and 2022 has been presented in Fig. 1 and 2.

Overall maximum severity of Marssonina blotch (32.31 %) was recorded under CFS in comparison to NFS (22.87 %). Among the natural orchards, maximum severity of Marssonina blotch (29.45 %) was recorded at location MD while, minimum (10.70 %) was recorded at location SJ. Among the chemical orchards, maximum

APPENDIX-I

Composition of Jeevamrit		
Ingredient		**Quantity for 200 lt water**
Cow dung	:	10 kg
Cow urine	:	5-10 lt
Jaggery	:	2 kg
Pulse flour	:	2 kg
Handful of soil		-

Composition of Ghanjeevamrit		
Ingredient		**Quantity for 200 lt water**
FYM	:	100 kg
Cow urine	:	3 lt
Jaggery	:	1 kg
Pulse flour	:	1 kg
Handful of soil		-

Composition of Bhramastra		
Ingredient		**Quantity for 200 lt water**
Cow urine	:	20 lt
Neem leaves	:	2 kg
Karanj leaves	:	2 kg
Custard apple leaves	:	2 kg
Castor leaves	:	2 kg
Datura leaves	:	2 kg

Composition of Sonthastra		
Ingredient		**Quantity for 200 lt water**
Cow milk	:	5 lt
Dry sonth	:	200 g

Composition of Agniastra		
Ingredient		**Quantity for 200 lt water**
Cow urine	:	200 lt
Neem leaves	:	2 kg
Tobacco powder	:	500 g
Green chilli powder	:	500 g
Garlic paste	:	250 g
Turmeric powder		200 g

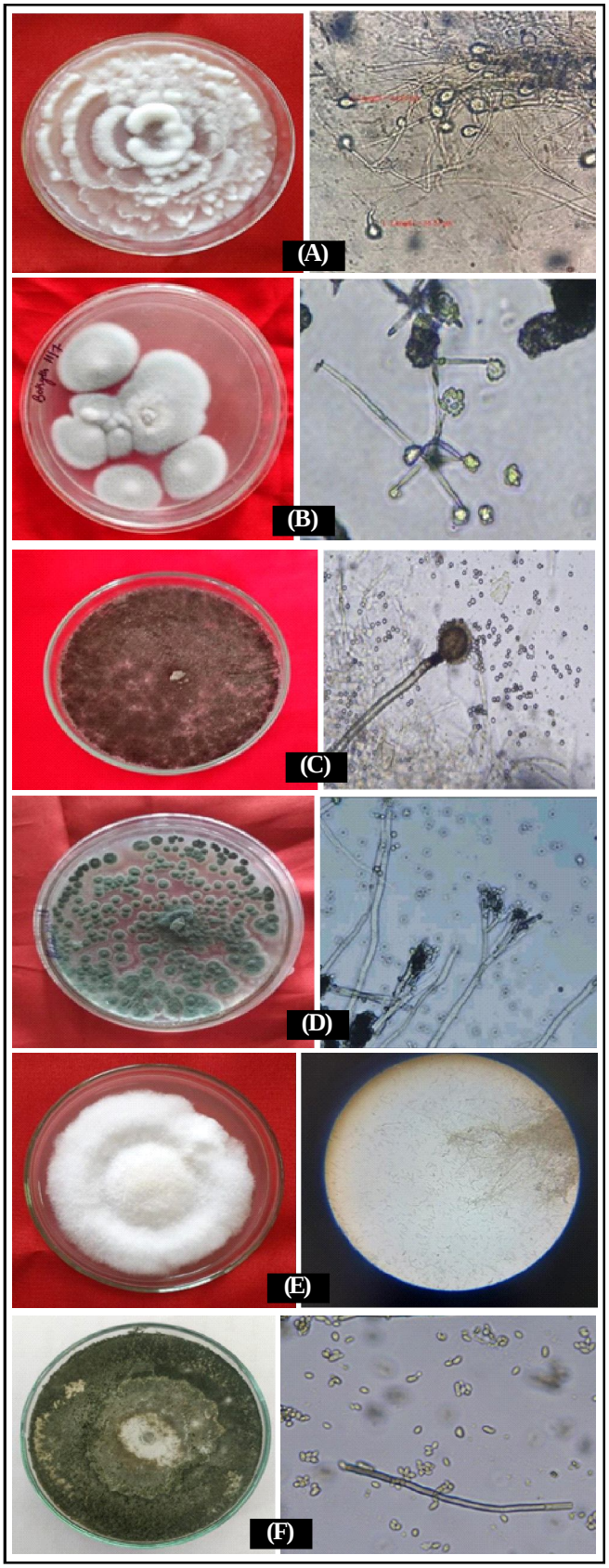

Fig. 5: Cultural and microscopic view of isolated fungal microflora (A- *Phytophthora* sp., B- *Botrytis* sp., C- *Mucor* sp., D- *Penicillium* sp., E- *Fusarium* sp., F- *Trichoderma* sp.).

Table 4: Comparative status of bacterial microflora in soils under natural and chemical farming systems of apple production.

Bacterial Count (cfu g^{-1} of soil)							
Location	NFS			CFS			Per cent increase over CFS
	Dilutions			Dilutions			
	10^5	10^6	Mean	10^5	10^6	Mean	
LG	25.50 (6.41)	24.50 (7.39)	**25.00 (6.90)**	20.50 (6.31)	16.00 (7.21)	**18.25 (6.76)**	27.00 (2.03)
SR	46.50 (6.67)	32.50 (7.52)	**39.50 (7.09)**	44.50 (6.65)	22.00 (7.34)	**28.25 (6.99)**	15.82 (1.41)
HM	43.50 (6.64)	33.50 (7.53)	**38.50 (7.08)**	38.00 (6.58)	12.00 (7.08)	**25.00 (6.83)**	35.06 (3.57)
MD	36.50 (6.56)	21.50 (7.33)	**29.00 (6.95)**	20.00 (6.30)	10.00 (7.0)	**15.00 (6.65)**	48.28 (4.28)
SJ	46.00 (6.66)	24.50 (7.39)	**35.25 (7.03)**	46.50 (6.67)	23.00 (7.37)	**33.75 (7.02)**	0.71 (0.11)
Mean	39.60 (6.59)	25.74 (7.39)	**33.45 (7.01)**	33.90 (6.50)	16.70 (7.20)	**24.05 (6.85)**	24.36 (2.28)
Figures in parentheses are log transformed values							

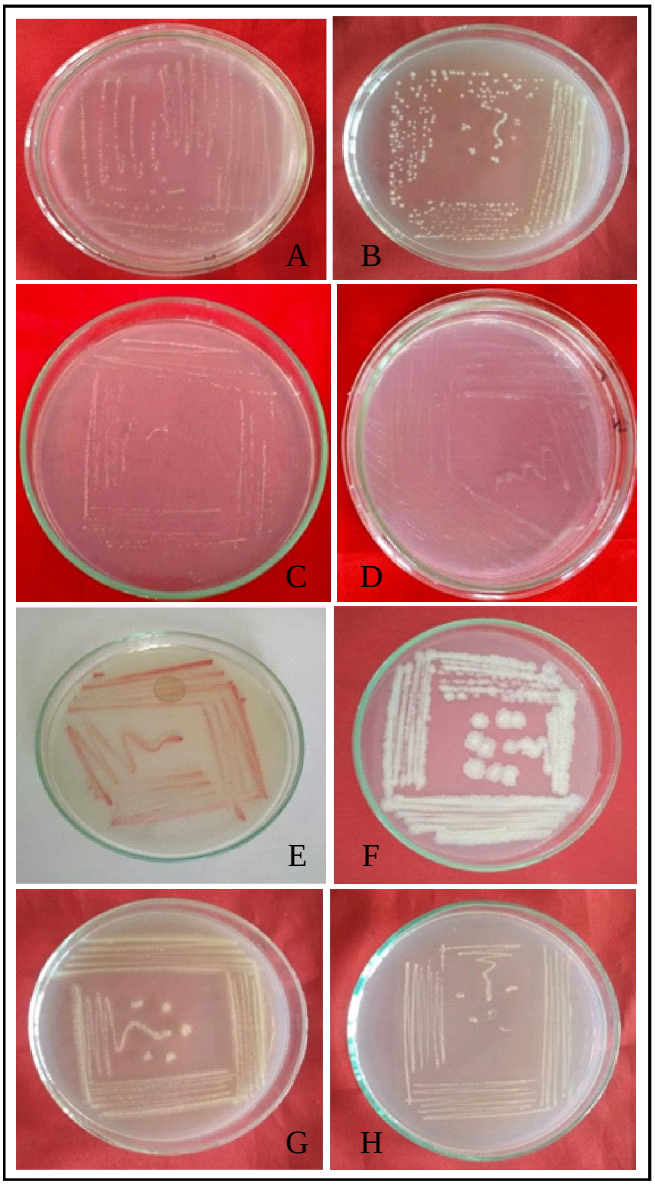

Fig. 6: Cultural view of isolated bacterial microflora (A- *Exiguobacterium aurantiacum*, B- *Bacillus cereus* strain 2R-A, C- *Staphylococcus sp.*, D- *Alcaligenes faecalis*, E- Serratia *liquefaciens*, F- *Bacillus cereus* group, G- *Pasteurella dagmatis*, H- *Ochrobactrum intermedium*.

severity of Marssonina blotch (38.65 %) was recoded at location LG while, minimum severity (15.50 %) was recorded at location SJ. Overall, per cent decrease over CFS was found to be -41.28 per cent, while it was found maximum (-98.21 %) at site LG among different sites.

Overall maximum severity of Alternaria leaf spot (16.42 %) was recorded under CFS in comparison to NFS (11.91 %). Among the natural orchards, highest severity (19.85 %) was recorded at location LG and lowest severity (7.35 %) was recorded at location SJ. Among chemical orchards, highest severity (26.95 %) was recorded at location MD and lowest severity (9.30 %) was recorded at location HM. Overall per cent decrease over CFS was found to be -37.90 per cent, while it was found maximum (-73.31 %) at site MD among different sites. Comparatively lower severity of diseases under NFS may be because of the use of natural formulations like jeevamrit and buttermilk from last 3 to 4 years. This may have resulted in change in natural microbiome ultimately activating the natural defense system of plants. Under NFS, farmers used to spray buttermilk to control diseases. The antifungal activity of buttermilk is attributed to the presence of lactic acid bacteria which produces antifungal metabolites such as proteinaceous compounds and fatty acids (Schniirer and Magnusson, 2005). Buttermilk has also been reported to reduce the severity of sorghum downy mildew (Haldhar *et al*., 2017). These results are also in conformity to a report released by NITI Ayog (HP) in 2020. They surveyed orchards under natural farming system in Shimla district of HP during 2018-19 and found lowest incidence of Marssonina blotch (12.20 %) in natural farming system in comparison to conventional farming system (18.40 %).

Comparative status of soil microbiome in NFS and CFS of apple production Fungal microflora

The fungal count was determined from collected soil samples by Serial Dilution Method and the results are presented in the Table 3. Overall fungal abundance was

Table 5: Characteristics of fungal microflora isolated from soils of study orchards.

S.	Isolate	Description	Identified as
1.	Isolate 1	Colony colour was white; colony grew up to 9 cm within 7-8 days of incubation at 25R°C; mycelium was aseptate for both the isolates and sporangia were lemon-shapedhaving dimensions 40×3 µm and 46×3.5 µm	*Phytophthora* sp.
2.	Isolate 2	Colony colour was greyish with white outline; colony grew in patches on PDA; mycelium was septate; conidiophore was observed with tree-like branches having dimensions of 40 µm; conidia were attached toconidiophore in grape-like bunches and was 2.9 µm in size	*Botrytis* sp.
3.	Isolate 3	Colony colour was black and within three days of incubation the colony grew fully in Petri plate; mycelium was aseptate and sporangia were globose with dimensionof 40×36.6 µm; sporangiospores (4.6×3.5 µm) were attached to sporangia in chains	*Mucor* sp.
4.	Isolate 4	Colony was dark green in colour; colony grew in patches on PDA. Under microscopic observation of colony, mycelium was septate and conidiophore bear brush likestructure called penicillus having dimensions 36 µm	*Penicillium* sp.
5.	Isolate 5	Colony was white in colour and puffy in appearance; mycelium was septate and two types of conidia- macroconidia and microconidia were seen; macroconidia were sickle-shaped and multicelled having dimensions of27.44×3.44 µm while microconidia were circular and single celled having dimensions of 6.3 µm	*Fusarium* sp.
6.	Isolate 6	Colony was white in colour; mycelium was septate andconidia (5×4 µm) were borne on phillade	*Trichoderma* sp.

found to be higher under NFS (34.45 cfu g^{-1} of soil) as compared to CFS (26.80 cfu g^{-1} of soil). Under NFS, location SR recorded maximum fungal count (66×10^3 and 32.5×10^4 cfu g^{-1} of soil) while LG recorded minimum fungal count (19.5×10^3 and 9.5×10^4 cfu g^{-1} of soil). Under CFS, location SJ recorded maximum fungal count (44.5×10^3 and 31.5×10^4 cfu g^{-1} of soil) while LG recorded minimum fungal count (11.5×10^3 and 6×10^4 cfu g^{-1} of soil). Overall, per cent increase of fungal count over CFS found to be 22.21 per cent, while maximum per cent increase was found at location LG (39.66 %) (Fig. 3).

Bacterial microflora

The bacterial count was determined from collected soil samples by Serial Dilution Method and the results are presented in the Table 4. Overall bacterial count was found to be higher under NFS (33.45 cfu g^{-1} of soil) as compared to CFS (24.05 cfu g^{-1} of soil). Under NFS, location SR recorded maximum bacterial count (46.5×10^5 and 32.5×10^6 cfu g^{-1} of soil) while LG recorded minimum

Table 6: Characteristics of bacterial microflora isolated from soils of study orchards.

	Colony Morphology				Shape	GR	Biochemical Tests						Identified as
	Form	Elevation	Margin	Colour			SAT	IT	CUT	OT	TST	CT	
I-1	Puncti-form	Convex	Entire	White	Rod	-	+	-	+	+	+	+	*Exiguobacterium aurantiacum*
I-2	Spindle	Pulvinate	Entire	Colour-less	Rod	-	+	-	+	-	+	-	*Bacillus cereus* strain 2R-A
I-3	Spindle	Pulvinate	Entire	Orange	Rod	+	-	-	+	-	+	+	*Staphylococcus sp.*
I-4	Spindle	Pulvinate	Entire	Pale Yellow	Rod	+	-	-	+	-	+	-	*Alcaligenes faecalis*
I-5	Puncti-form	Umbonate	Lobate	White	Rod	+	-	-	+	+	-	-	Serratia *liquefaciens*
I-6	Spindle	Pulvinate	Entire	Colour-less	Spiral	-	-	-	-	+	-	-	*Bacillus cereus* group
I-7	Irregular	Umbonate	Lobate	White	Spherical	+	+	-	+	-	+	-	*Pasteurella dagmatis*
I-8	Circular	Convex	Entire	Red	Circular	+	-	-	-	+	-	+	*Ochrobactrum intermedium*

I: Isolate; **GR:** Gram Reaction; **SAT:** Starch Amylase Test; **IT:** Indole Test; **CUT:** Citrate Utilization Test; **OT:** Oxidase Test; **TST:** Triple Sugar Test; **CT:** Catalase Test

bacterial count (25.5×10^5 and 24.5×10^6 cfu g^{-1} of soil). Under CFS, location SJ recorded maximum bacterial count (46.5×10^5 and 23.0×10^6 cfu g^{-1} of soil) while LG recorded minimum bacterial count (20.5×10^5 and 16.0×10^6 cfu g^{-1} of soil). Overall, per cent increase of bacterial count over CFS found to be 24.36 per cent, while maximum per cent increase was found at location MD (48.28 %) (Fig. 4).

These results are in consistence with Lavanya *et al.*, (2016). They reported that after application of jeevamrit in bean field, there was increase in bacterial and fungal population from 5×10^5, 3×10^3 to 40×10^5, 32×10^3 cfu g^{-1} of soil, respectively. Joshi (2008) also reported that application of jeevamrit increases microbial population in soil. Also, application of different concentrations of panchgavya increased microbial activity as compared to FYM and vermicompost (Jain *et al.*, 2014).

Basal respiration and microbial biomass were recorded to be higher in organic apple farms (Bougnom *et al.*, 2012). The increase of microbial activity and biomass can be attributed to the incorporation of easily degradable materials, entering the soil from different organic wastes stimulating the indigenous microbial activity of soil (Fuchs, 2009).

Purification and identification of isolated microflora

In total, 6 fungal (Fig. 5) and 08 bacterial (Fig. 6) isolates were purified, the details of which has been presented in Table 5 and 6. Fungal isolates were identified based on cultural and morphological characters. For bacterial isolates, cultural, morphological, and biochemical characteristics were studied. The bacterial antagonists were identified as *Exiguobacterium aurantiacum*, *Bacillus cereus* strain 2R-A, *Staphylococcus sp.*, *Alcaligenes faecalis*, Serratia *liquefaciens*, *Bacillus cereus* group, *Pasteurella dagmatis* and *Ochrobactrum intermedium* by sending their pure cultures to CSIR-NCIM Pune, Maharashtra and Biokart Genomic Laboratory, Bengaluru, Karnataka.

Conclusion

Both natural and chemical farming systems have their respective advantages and challenges in apple production. Natural farming supports a healthier soil microbiome and fosters long-term sustainability but may struggle with higher disease incidence initially. Conventional farming effectively controls foliar diseases but can negatively impact soil health and microbial diversity, posing long-term ecological risks. Balancing these systems through integrated pest management and soil health practices could provide a sustainable approach to apple farming.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

Data will be provided on request.

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