In vitro bio-efficacy of plant essential oils against major fungal and bacterial diseases of cauliflower

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ABSTRACT

The study was conducted to evaluate the in vitro efficacy of seven plant essential oils against major fungal and bacterial pathogens causing significant diseases in cauliflower: *Sclerotinia sclerotiorum* (stalk rot), *Alternaria brassicicola* (alternaria leaf spot) and *Xanthomonas campestris* pv *campestris* (black rot). The essential oils tested included *Mentha arvensis, Eucalyptus hybrid, Rosmarinus officinalis, Melia azedarach, Syzygium aromaticum, Tagetes minuta* and *Cinnamomum camphora*, evaluated at concentrations ranging from 25 to 250 ppm using poisoned food technique for fungi and inhibition zone assay for bacteria. Results showed varied inhibitory effects among the oils. *C camphora* demonstrated the highest mycelial growth inhibition against *S sclerotiorum* (83.06%) and *A brassicicola* (82.50%). For the bacterial pathogen, *X campestris* pv *campestris*, *S aromaticum* exhibited the maximum inhibitory effect (34.33 mm inhibition zone) followed closely by *M azedarach* and *C camphora*. These findings highlight the potential of certain plant essential oils as promising bio-fungicides and bio-bactericides, offering eco-friendly alternatives for the sustainable management of cauliflower diseases, thereby, reducing reliance on synthetic chemicals and addressing residue concerns.

Keywords: Cauliflower; plant essential oils; *Sclerotinia sclerotiorum*, *Alternaria brassicicola*, *Xanthomonas campestris* pv *campestris*, in vitro efficacy; disease management

INTRODUCTION

Cauliflower (*Brassica oleracea* var *botrytis* L), a member of family Cruciferae, is the most important cole crop grown for both curd and seed purposes all over the world. It occupies a pride place among cole crops due to its delicious taste, flavour and nutritive value. Cauliflower has high quality proteins and peculiar in stability of vitamin C after cooking. It is rich in minerals. Its fresh curd is highly nutritive and contains moisture 90.8 g, protein 2.6 g, fat 0.4 g, minerals 1.0 g, fiber 1.2 g, carbohydrates 4.0 g, energy 30 kcal, calcium 33 mg, phosphorus 57 mg, Iron 1.5 mg, carotene 30 mg, thiamine 0.04 mg, riboflavin 0.10 mg, niacin 1.0 mg and vitamin C 56.0 mg per 100 g of edible portion (Jood and Khetrapaul 2011).

Cauliflower in Himachal Pradesh is cultivated on an area of 5.92 thousand hectares with a production of 139.14 thousand metric tonnes (Lalenpuii et al 2025). Due to varied agro-climatic conditions, it is grown throughout the year in different parts of the state for table (curd) as well as seed purposes. Due to extensive and continuous cultivation of Snowball type of cultivars for curd and seed purpose, the cauliflower production in Himachal Pradesh has suffered a lot. Though the area and production under cauliflower cultivation in this state has increased during the last few decades but the productivity per unit area has not increased proportionally and is quite low as compared to the advanced cauliflower growing countries of the world. One of the reasons is surely the damage caused by insect pests and a variety of fungal, bacterial and viral diseases, which not only reduce the quantity but also hamper the quality of the produce. Of late, the most important fungal and bacterial diseases which affect its production include black rot {*Xanthomonas campestris* pv*campetris* (Pammel) Dowson},

Sclerotinia rot {*Sclerotinia sclerotiorum* (Lib) de Bary} and *Alternaria* leaf spot (*Alternaria brassicicola* Schwein). *Sclerotinia* rot is one of the destructive fungal diseases of cauliflower in relatively cool and moist areas affecting both field and seed crops (Saharan and Mehta 2008). The disease was reported to cause the serious losses in the different regions of the world on different crops. The disease was first noticed in 1973 in a few isolated locations in Saproon valley of Himachal Pradesh but has since increased very rapidly (Banik and Sharma 2009).

Black rot of cauliflower incited by *X campestris* pv *campestris* is one of the most devastating bacterial diseases of cauliflower and other vegetable brassicas reported from all over the world. The disease causes considerable yield losses in the field as well as in the seed crops worldwide (Williams 1980). In India, the disease was recorded for the first time on cauliflower by Patel et al (1949) and subsequently it was reported from Katrain (Kullu) area of Himachal Pradesh (Rao and Srivastava 1964). The crop losses ranging from 10-100 per cent have been reported in cauliflower (Dhar and Singh 2014, Kashyap and Dhiman 2010). Alternaria leaf spot is the most common and serious menace to the cultivation of cruciferous vegetables world over (Kear et al 1977). Both *A brassicicola* and *A brassicae* have been reported to cause the leaf spot on cauliflower, almost from every continent. In India, the disease was first reported by Butler (1918) in mustard and later the organism was identified as *A brassicae*. On cauliflower, broccoli and other brassicas, more than 50 per cent yield losses have been reported (Verma and Saharan 1994, Sharma et al 2013). Due to the heavy use of chemicals viz fungicides and antibiotics for the management of these major fungal and bacterial diseases of cauliflower, there are residual issues in the curd and toxicity to the humans when not consumed at a recommended days of waiting period. Thus in the present study, management of the pathogens through plant oils as an alternative was carried out to evaluate the efficacy under in vitro conditions.

MATERIAL and METHODS

Isolation of the fungal and bacterial pathogens

Infected leaves, stalks and curds, exhibiting characteristic symptoms of fungal diseases, were brought to the laboratory for isolation of the pathogens by following the standard methods of isolation of fungal and bacterial pathogens. Cultures so obtained were purified by hyphal tip method and identified based on morphological and taxonomic characters. Cultures were maintained on potato dextrose agar medium at 4±1°C. For isolation of the black rot pathogen, 4-5 infected bits were surface-sterilized with 0.1 per cent mercuric chloride and placed in the sterilized distilled water in a flask under aseptic condition and later kept over the shaker for 24 hours in order to obtain the bacterial ooze. A loopful of bacterial suspension was taken from the flask and streaked on nutrient agar medium plates under aseptic conditions. The Petri plates were incubated at 30°C for 72 hours and observed for colony formation of the pathogen. For purification and maintenance of the bacterial culture, the circular, yellow and mucoid colonies of the bacterium were picked from the Petri plates and transferred to Petri plates containing the Luria Bertani (LB) medium. These cultures were further purified by streak plate method and maintained on LB medium slants at 5°C for further studies. The cultures were periodically sub-cultured at fortnight intervals. The bacterial pathogen was identified on the basis of morphological and cultural characters along with Gram staining (Plate 1).

In vitro evaluation of plant oils

In order to judge the efficacy of seven plant oils against *S sclerotiorum*, *A brassicicola* and *X campestris* pv *campestris,* seven different plant oils viz *Mentha arvensis, Eucalyptus hybrid, Rosmarinus officinalis, Melia azedarach, Syzygium aromaticum, Tagetes minuta* and *Cinnamomum camphora* were used in this study. The oils were purchased from the Department of Forest Products, College of Forestry, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh and evaluated under in vitro conditions by poisoned food technique (Falck 1907) to study the inhibitory effect of these oils on the mycelial growth of *S sclerotiorum* and *A brassicicola*. While to study the antibacterial effect of plant oils against *X campestris* pv *campestris* under in vitro conditions, inhibition zone assay method (Vincent 1947) was followed. Four different concentrations of the plant extracts 25, 50, 100 and 250 ppm were used. Observations were recorded on diametric mycelial growth (mm)/inhibition zone (mm).

RESULTS and DISCUSSION

Effect of essential plant oils

Data on the effect of seven essential oils on diametric mycelial/colony growth and

per cent growth inhibition are detailed in Tables 1, 2 and 3.

The data presented in the Table 1 (Plate 2) reveal that the test plant oils provided 0.00 to 83.06 per cent inhibition of *S sclerotiorum* mycelial growth with *C camphora* showing the maximum inhibitory effect (83.06%) followed by *S aromaticum* (71.67%).

The next best treatments included *R officinalis* and *E hybrida* oils which provided 32.59 and 1.39 per cent growth inhibition respectively. *M arvensis, T minuta* and *M azedarach* oils were, however, the least effective exhibiting nil mycelial growth inhibition of the test pathogen.

The data presented in Table 2 (Plate 3) reveal that the test plant oils provided 0.00 to 82.50 per cent inhibition of *A brassicicola* mycelial growth with *C camphora* showing the maximum inhibitory effect (82.50%) followed by *Syzygium aromaticum* (73.89%).

The next best treatments included *M azedarach*, *E hybrida, M arvensis* and *T minuta* oils which provided 31.30, 11.67, 11.20 and 8.52 per cent growth inhibition respectively. *R officinalis* oil was, however, the least effective exhibiting no mycelial growth inhibition of the test pathogen.

The data presented in Table 3 (Plate 4) highlights that the test plant oils provided 16.34 to 34.33 mm average zone inhibition of *X campestris* pv *campestris* with *S aromaticum* showing the maximum inhibitory effect (34.33 mm) followed by *M azedarach* (30.67 mm) and *C camphora* (30.33%), the latter two being at par. The next best treatments included *E hybrida, R officinalis* and *T minuta* oils which provided 29.68, 25.68 and 21.34 mm zone inhibition respectively. *M arvensis* oil was, however, least effective exhibiting 16.34 mm zone inhibition of the test bacterial pathogen.

Some studies have documented satisfactory results in using essential oils of some plants against *S sclerotiorum* for example rosemary (*R officinalis*) (Pitarokili et al 2008) and neem (*Azardirachta indica*) (Moslem and El-Kholie 2009). Barman et al (2015) used seven different plant oils against *A alternata* causing leaf blight of tomato and reported that eucalyptus oil and neem oil @ 0.05 and 0.1 per cent resulted in 97.2 and 95.1 per cent inhibition of mycelial growth of the pathogen. Lucas et al (2012) reported that concentrations above 10 per cent of the essential oils of cinnamon, clove, citronella, tea tree, lemon

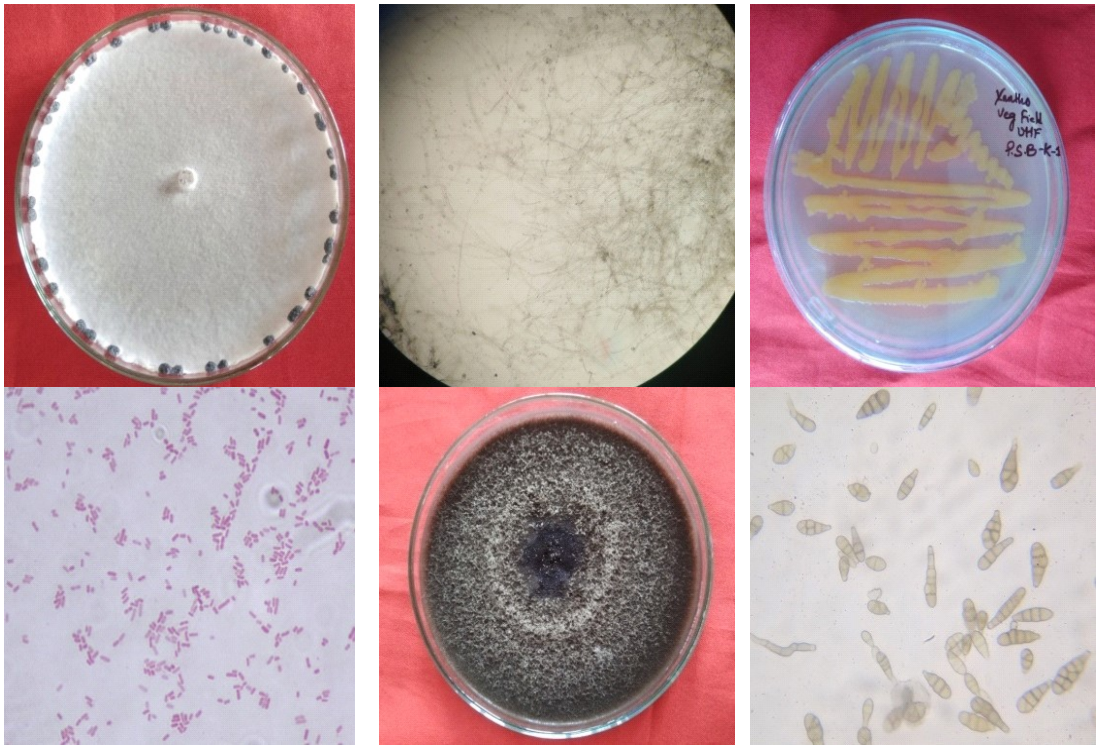

Plate 1. Morphological and cultural characteristics of pathogens causing stalk rot, black rot and *Alternaria* leaf spot of cauliflower

Table 1. Effect of plant oils on mycelial growth inhibition of *Sclerotinia sclerotiorum* causing stalk rot of cauliflower

Plant oil	Average mycelial growth (mm)					Average mycelial growth inhibition (%)				
	Concentration (ppm)				Mean	Concentration (ppm)				Mean
	25	50	100	250		25	50	100	250	
Mentha arvensis	90.00	90.00	90.00	90.00	90.00	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)
Eucalyptus hybrida	89.67	89.00	88.67	87.67	88.75	0.37 (3.10)	1.11 (5.41)	1.48 (6.89)	2.59 (9.21)	1.39 (6.15)
Rosmarinus officinalis	89.33	56.00	54.67	42.67	60.67	0.74 (2.86)	37.78 (37.91)	39.26 (38.78)	52.59 (46.47)	32.59 (31.50)
Cinnamomum camphora	24.33	14.00	12.00	10.67	15.25	72.96 (58.65)	84.44 (66.75)	86.67 (68.57)	88.15 (69.85)	83.06 (65.96)
Syzygium aromaticum	90.00	12.00	0.00	0.00	25.50	0.00 (1.62)	86.67 (68.57)	100.00 (88.34)	100.00 (88.34)	71.67 (61.72)
Tagetes minuta	90.00	90.00	90.00	90.00	90.00	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)
Melia azedarach	90.00	90.00	90.00	90.00	90.00	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)
Mean	80.48	63.00	60.76	58.71		10.58 (10.15)	30.00 (29.63)	32.49 (29.63)	34.76 (31.25)	

Angular transformed values within parentheses

$CD_{0.05}$

	Mycelial growth	Mycelial growth inhibition
Plant oil	0.59	1.13
Concentration	0.45	0.86
Plant oil × Concentration	1.19	2.27

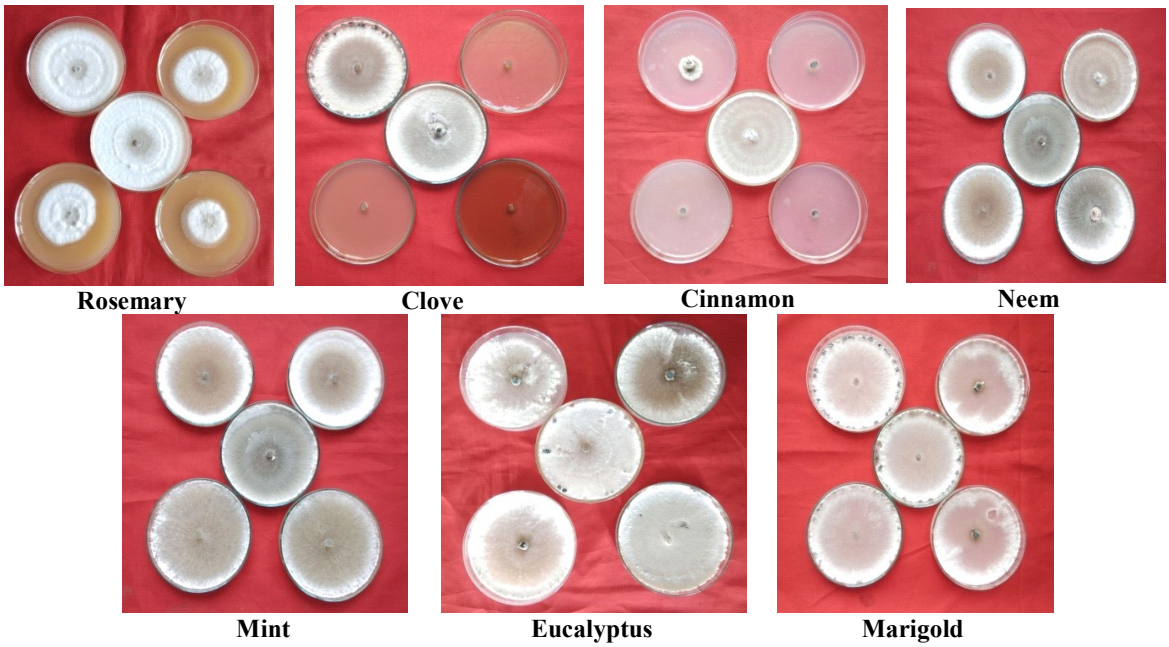

Plate 2. In vitro inhibition of *Sclerotinia sclerotiorum* causing stalk rot of cauliflower by plant oils

Table 2. Effect of plant oils on mycelial growth inhibition of *Alternaria brassicicola* causing leaf spot of cauliflower

Plant oil	Average mycelial growth (mm)					Average mycelial growth inhibition (%)				
	Concentration (ppm)				Mean	Concentration (ppm)				Mean
	25	50	100	250		25	50	100	250	
Mentha arvensis	83.33	80.67	78.67	77.00	79.92	7.41 (15.72)	10.37 (18.76)	12.59 (20.76)	14.44 (22.32)	11.20 (19.39)
Eucalyptus hybrida	83.33	81.67	80.00	73.00	79.50	7.41 (15.72)	9.26 (17.66)	11.11 (19.45)	18.89 (25.75)	11.67 (19.64)
Rosmarinus officinalis	90.00	90.00	90.00	90.00	90.00	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)	0.00 (1.62)
Cinnamomum camphora	36.33	26.67	0.00	0.00	15.75	59.63 (50.54)	70.37 (67.53)	100.00 (88.34)	100.00 (88.34)	82.50 (73.69)
Syzygium aromaticum	48.33	33.00	12.67	0.00	23.50	46.30 (42.86)	63.33 (52.72)	85.93 (67.95)	100.00 (88.34)	73.89 (62.97)
Tagetes minuta	90.00	90.00	76.33	73.00	82.33	0.00 (1.62)	0.00 (1.62)	15.19 (22.91)	18.89 (25.75)	8.52 (12.97)
Melia azedarach	77.00	71.00	69.67	29.67	61.84	14.45 (22.26)	21.11 (27.34)	22.59 (28.37)	67.04 (54.98)	31.30 (33.24)
Mean	72.62	67.57	58.19	48.95		10.58 (10.15)	30.00 (29.63)	32.49 (29.63)	34.76 (31.25)	

Angular transformed values within parentheses

$CD_{0.05}$

	Mycelial growth	Mycelial growth inhibition
Plant oil	1.23	2.97
Concentration	0.93	2.24
Plant oil × Concentration	2.46	5.94

Table 3. In vitro evaluation of different plant oils against *Xanthomonas campestris* pv *campestris* causing black rot of cauliflower

Plant oil	Average inhibition zone (mm)
Mentha arvensis	16.34
Eucalyptus hybrida	29.68
Rosmarinus officinalis	25.68
Cinnamomum camphora	30.33
Syzygium aromaticum	34.33
Tagetes minuta	21.34
Melia azedarach	30.67
$CD_{0.05}$	0.80
SE(m)	16.33

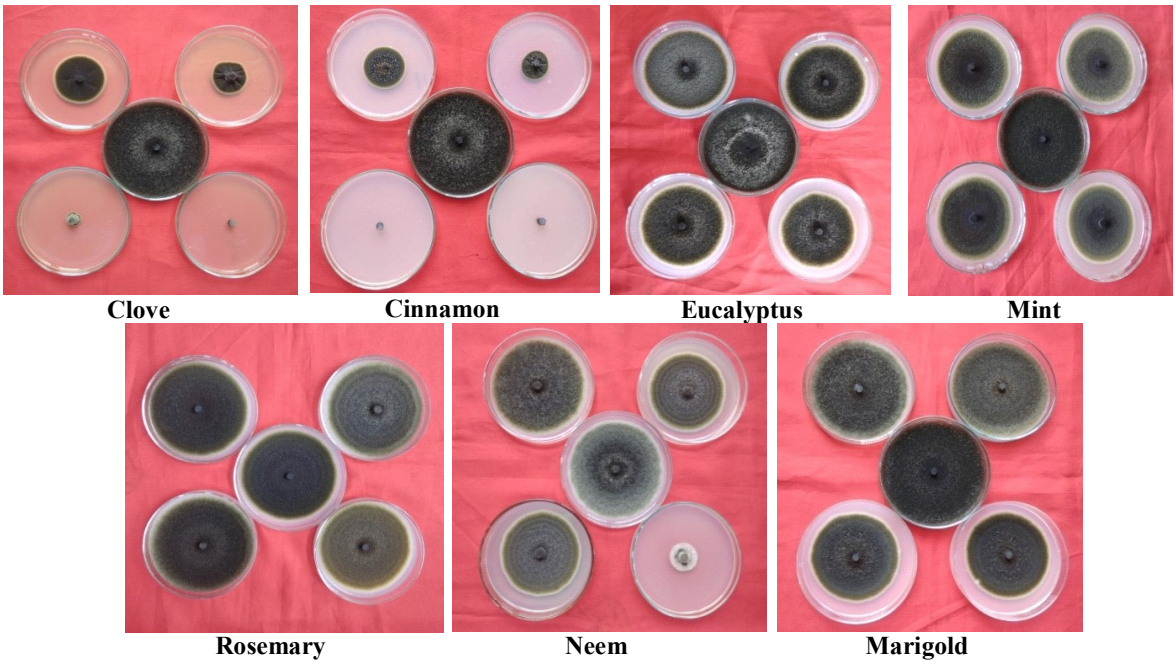

Plate 3. In vitro inhibition of *Alternaria brassicae* causing leaf spot of cauliflower by plant oils

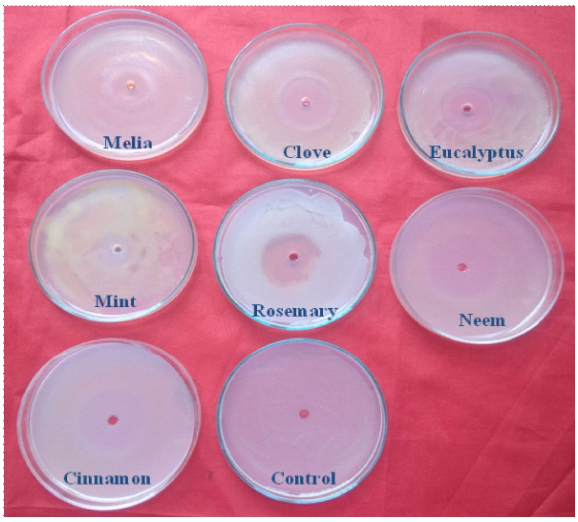

Plate 4. In vitro evaluation of plant oils against *Xanthomonas campestris* pv *campestris* causing black rot of cauliflower

grass, thyme and eucalyptus were effective for in vitro growth inhibition of *X vesicatoria.*

CONCLUSION

This in vitro study conclusively demonstrates the significant fungicidal and bactericidal potential of various plant essential oils against key cauliflower pathogens: *Sclerotinia sclerotiorum* (stalk rot), *Alternaria brassicicola* (*Alternaria* leaf spot) and *Xanthomonas campestris* pv *campestris* (black rot). Among the tested oils, *Cinnamomum camphora* consistently exhibited the highest inhibitory effect on the mycelial growth of both *S sclerotiorum* (83.06%) and *A brassicicola* (82.50%). For the bacterial pathogen, *X campestris* pv *campestris*, *Syzygium aromaticum* proved most effective with the largest inhibition zone (34.33 mm) followed closely by *Melia azedarach* and *C camphora*. These findings strongly suggest that plant essential oils, particularly *C camphora* and *S aromaticum*, hold promise as environmentally friendly and sustainable alternatives to synthetic chemical pesticides for managing major diseases in cauliflower. Their efficacy under in vitro

conditions warrants further investigations through in vivo trials to validate their performance in field settings and develop practical application strategies for integrated disease management programmes in Himachal Pradesh and other cauliflower-growing regions. This approach could significantly mitigate concerns regarding chemical residues and environmental toxicity, promoting safer and healthier produce.

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