Efficacy of botanicals against *Pestalotia* leaf spots in strawberry

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ABSTRACT

Pestalotia leaf spot is a serious disease of strawberry in temperate and subtropical areas. In the present study, out of thirteen aqueous botanicals evaluated under in vitro conditions, neem (*Azadirachta indica*) and Darek (*Melia azedarach*) were found most effective with 38.26 and 38.10 per cent of mycelial inhibition of *Pestalotia* followed by *Roylea elegans* (34.56%) and *Ocimum sanctum* (33.82%). The mycelial growth inhibition increased with the increase in concentration of plant extract. Among botanicals-based bio-formulations, Neemazal was found most effective with 75.78 per cent mycelial inhibition followed by botanical field formulation-2 (BFF-2) (57.05%) and botanical field formulation-1 (BFF-1) (26.16%). Among botanicals-based bio-formulations, Neemazal and BFF-2 were found most effective with disease incidence of 10.38 and 12.06 per cent and disease index of 4.24 and 6.54 per cent respectively. The yield recorded was 137.45, 141.34 and 138.00 q/ha and number of runners was 36.0, 37.6 and 35.8 in BFF-1, BFF-2 and Nemazal treatments respectively which were higher than control (53.68 q/ha and 13.8 respectively) but there were no differences among BFF-1, BFF-2 and Nemazal for these traits. Highest average plant height of 39.34 cm was recorded in the treatment BFF-2 followed by BFF-1 and Neemazal with plant height of 37.86 and 36.72 cm respectively, the latter two being at par. Thus foliar sprays of cow urine-based botanical field formulation (BFF-2) at 10 days interval starting from first week of July were found effective and may be recommended for management of disease under field conditions.

Keywords: *Pestalotia* leaf spot; strawberry; botanicals; disease incidence

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch) is an important fruit of the family Rosaceae. The cultivated strawberry is a result of the hybridization of two American species of the crop (*Fragaria* × *chiloensis* and *F* × *virginiana*) and is an octaploid (2n = 56). Strawberry fruits are attractive with pleasant aroma and good flavour and are also rich in vitamin C (39 to 86 mg/100 g fruit) and minerals. The flavour of the fruit is attributed to the presence of volatile esters which vary among cultivars. The fruits are rich in ascorbic acid, secondary metabolites and simple sugars (Kafkas et al 2007).

Strawberry fruits are richest source of bioactive phytochemicals with potential antioxidant activities, mainly ellagic acid and flavonoids which can lower the risk of cardiovascular diseases and tumorogenesis (Wang et al 1996, Heinonen et al 1998). Strawberry is a non-climacteric fruit and its ripening is accompanied with changes in colour, texture and flavour that give the fruit its unique characteristics. It prefers slightly acidic soils. Strawberry fruits have a special demand from fruit processing industries for preparing jam, jellies, candies, drinks etc.

Strawberry is grown throughout the world up to an elevation of 3,000 m amsl in humid and dry regions (Darrow and Waldo 1934). Worldwide 9,125,913 tonnes of strawberry is produced per year. China is the largest strawberry producer in the world with 3,801,865 tonnes production volume per year (https://www.atlasbig.com/en-in/countries-by-strawberry-production). In India, total area under strawberry cultivation is around 1,000 ha with an annual production of 5,000 MT (Anon

2018). It is cultivated in Jammu and Kashmir, Himachal Pradesh, Maharashtra, West Bengal, Nilgiri Hills, Haryana, Punjab and some parts of Delhi. With the introduction of low chilling and day neutral cultivars, strawberry cultivation is gaining more popularity in the plains of Punjab and northern states due to high yield in the crop and remunerative returns.

Strawberry is infected by a number of diseases caused by fungi, bacteria, viruses and other pathogens. Amongst fungal diseases, grey mould (*Botrytis cinerea*), crown and leather rot (*Phytophthora cactorum*), red stele (*Phytophthora fragariae*), anthracnose (*Colletotrichum acutatum*), southern blight (*Sclerotium rolfsii*), leaf spots (*Mycosphaerella fragariae*), leaf blight (*Phomopsis obscurans*), leaf scorch (*Diplocarpon earlianum*) and leaf spots caused by different species of *Pestalotia* (*Pestalotia laurocerasi* and *P longisetula*) are important diseases which cause huge losses to the crop.

Leaf spots caused by fungal pathogens are among the serious problems which severely affect the growth and yield of strawberry. Among these, spots caused by *P laurocerasi* and *P longisetula* are important. *Pestalotia* leaf spots have been reported from many parts of the world. Incidence of *P longisetula* was reported from diseased strawberry in China (Zhu et al 1994). Camili et al (2002) also reported *P longisetula* causing strawberry rot from Brazil. *Pestalotia* spp have been reported as the causal agents of strawberry leaf spots disease in India (Bose 1970). *Pestalotia* rot of strawberry fruits caused by *P longisetula* was also reported by Shitole et al (2000) in India.

The symptoms of *Pestalotia* leaf spots start from the beginning of the rainy season in the form of grey or dirty-white spots measuring up to 15 mm in diameter. These spots increase in size and coalesce often involving most of the leaf surface. The fungus fructifies on the surface of the affected leaves forming concentric rings of minute, dot like, black structures. Later on, shot-holes are usually formed in the center of the spots and very often the entire central portion of the spot disintegrates and falls.

There is need for alternative methods of disease management in strawberry as use of chemical fungicides results in retention of residues in the fruits due to succulent nature of the latter. Use of botanical pesticides is one of the best alternatives for the management of foliar pathogens in strawberry (Stangarlin et al 2011). Botanical pesticides have been reported to be effective against leaf spots caused by *Pestalotia* in different crops (Rana et al 1999, Sindhan et al 1999, Hu et al 2001).

MATERIAL and METHODS

In vitro evaluation of botanicals

Disease management studies were done first by evaluating the plants with anti-fungal properties against the test pathogen under in vitro conditions and then making the formulations of effective botanicals.

Botanical formulations were evaluated under in vitro conditions along with commercial neem formulation (Neemazal) and recommended fungicides. Water extract of leaves from thirteen plants namely *Azadirachta indica* A Juss (neem), *Ocimum sanctum* L (Tulsi), *Vitex negundo* L (Banna), *Aloe vera* (L) Burm f (Gharitkumari), *Cymbopogon citratus* (DC) Stapf (lemon grass), *Lantana camara* L (lantana), *Eucalypyus* hybrid L (Safeda), *Mentha piperita* L (Pudina), *Withania somnifera* L Dunal (Ashwagandha), *Roylea elegans* Wall ex Benth (Kadu), *Cannabis sativa* L (Hemp), *Artemisia vulgaris* L (artemisia) and mature seeds of *Melia azedarach* L (Darek) were evaluated at different concentrations (10, 25 and 50%) under in vitro conditions against the *Pestalotia* leaf spot pathogen.

Sixty days old leaves of the plants were taken for making the water extract of different plant species except *M azedarach* where mature seeds of light yellow colour were taken for making the water extract. The extracts were tested by poisoned food technique (Falck 1907) to observe the inhibitory effect of these extracts on the mycelial growth of *P longisetula.*

Preparation of plant extracts

Sixty days old freshly harvested (200 g) leaves of each plant and seeds of *M azedarach* were taken. Leaves/seeds were washed under tap water. Each sample was grinded in mixer and blender by adding small quantity of sterilized distilled water to help in grinding. After grinding, 200 ml distilled water was added and homogenized in orbital shaker at 2,000 rpm for half an hour to get 100 per cent extract. The plant material was filtered through double-layered muslin cloth. Sterilization of the extract of different plants was done in an autoclave at 5 psi pressure for 30 minutes for 3 consecutive days and the extracts were kept in a refrigerator for further use.

In vitro efficacy of botanicals against the *Pestalotia* leaf spot pathogen

Evaluation of botanical extracts was done by poisoned food technique. Botanical extracts were tested at 10, 25 and 50 per cent concentration. Evaluation of the extracts was done at 50 per cent concentration by incorporating 50 ml of extract of the botanical at 100 per cent concentration in each case in 50 ml sterilized (autoclaved at 1.05 kg/cm^2 for 20 minutes) double strength melted PDA medium to get the 50 per cent concentration.

These mixtures of the media poisoned with different botanicals were cooled and poured in the sterilized Petri plates under aseptic conditions. Similarly, 50 ml of 50 per cent concentration of the water extract of botanicals was added in 50 ml of double strength PDA to get the 25 per cent concentration of the final mixtures. Ten ml of 100 per cent water extract of the botanicals was added in 90 ml of double strength PDA medium to get the desired 10 per cent concentration of the final mixture. A small culture bit of 5 mm size of the test pathogen (*P longisetula*) cut with a sterile cork borer was picked up with the help of sterilized inoculation needle and placed at the center of each Petri plate under aseptic conditions under laminar air flow work station.

Petri plates poured with double strength PDA medium and inoculated with the pathogen served as control. Each treatment was replicated thrice under completely randomized design (CRD). Petri plates were then incubated at 27 ± 1ºC for a period of 10-12 days. Growth of the mycelium was observed regularly and was measured as per cent inhibition by the formula given by Vincent (1947):

$$I = \frac{C - T}{C} \times 100$$

where I = Per cent mycelial inhibition; C = Diametric mycelial growth in control (mm); T = Diametric mycelial growth in treatment (mm)

Preparation and evaluation of botanical field formulations

Two botanical formulations (BFF-1 and BFF-2) made out of the effective botanicals were also evaluated under in vitro conditions. Out of thirteen plant extracts evaluated for their in vitro efficacy in inhibiting the growth of *Pestalotia* leaf spot pathogen, five most effective botanicals viz neem (*A indica*), Tulsi (*O sanctum*), lemon grass (*C citratus*), Kadu (*R elegans*) and Darek (*M azedarach*) were selected for making these botanical formulations.

Botanical formulations were made by taking 100 g of sixty days old freshly harvested leaves of neem, Tulsi, lemon grass and Kadu and 100 g seeds of Darek. These leaves and seeds were washed under the running tap water and with distilled water. The paste of all these ingredients was made in a mixer and grinder. In case of BFF-1, equal quantity of distilled water (100 × 5 = 500 ml) was added to this paste of leaves and seeds of five plants on weight and volume basis (w/v). In case of BFF-2, 500 ml cow urine of Desi (local) cow was added to the 500 g paste of the five botanicals.

In vitro evaluation of botanical field formulations and Neemazal

Evaluation of botanical formulations (BFF-1 and BFF-2) and commercial neem formulation (Neemazal) was done by poisoned food technique. Botanical formulations viz BFF-1 (water-based) and BFF-2 (cow urine-based) were prepared by mixing equal quantity of five best effective botanicals viz neem, Tulsi, lemon grass, Kadu and Drake. Botanical formulations (BFF-1 and BFF-2) were tested at 5, 10 and 25 per cent concentrations and commercial neem formulation (Neemazal) was tested at 1, 2 and 5 per cent concentrations.

Evaluation of both the formulations at 50 per cent concentration was done by incorporating 50 ml of the BFF-1 or BFF-2 at 100 per cent concentration in each case in 50 ml sterilized (autoclaved at 1.05 kg/cm for 20 minutes) double strength melted PDA medium to get the 50 per cent concentration. These mixtures of the media poisoned with different botanical formulations and commercial neem formulation were cooled and poured in the sterilized Petri plates under aseptic conditions.

A small culture bit of 5 mm size of the test pathogen (*P longisetula.*) cut with a sterile cork borer was picked up with the help of sterilized inoculation needle and was placed at the center of each Petri plate under aseptic conditions under laminar air flow. Petri plates poured with double strength PDA and inoculated with the pathogen served as control. Each treatment was replicated thrice under completely randomized design (CRD). Petri plates were then incubated at 27 ± 1ºC for a period of 10-12 days. Growth of the mycelium was observed regularly and was measured

as per cent inhibition by the formula given by Vincent (1947).

Field evaluation of botanicals

Disease management studies were carried out at the experimental farm of Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The experiment was conducted in randomized block design in 1 × 1 m^2 plot size. Distance from plant to plant and row to row was 45 cm × 45 cm. Plants of Chandler variety were used in the field experiment. Two botanical field formulations (BFF-1 and BFF-2) and Neemazal (commercial neem formulation) were evaluated under field conditions.

Botanical field formulation-1 (water-based), botanical field formulation-2 (cow-urine-based) were evaluated at 10 per cent concentration and commercial neem formulation (Neemazal) was evaluated at 1 per cent concentration. Sprays of different treatments were given starting with the appearance of the disease and the control plants were sprayed with water only. Ten sprays of each treatment were given by a foot sprayer starting from the first week of July at 10 days interval during 2018.

After the sprays, disease incidence and severity were recorded under each treatment. Disease incidence was recorded by counting the infected plants in a field in each treatment and disease index was recorded on five plants randomly selected per replication. In each replication, number of infected plants was counted and per cent disease incidence was calculated. Per cent disease incidence was calculated by the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{Disease index (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Yield (q/ha), height of the plants from bottom to the top apex of the plant (cm) and number of runners per plant were also recorded under each treatment.

RESULTS and DISCUSSION

In vitro evaluation of botanicals

The data on diametric growth of fungus were recorded and mycelial growth inhibition was calculated (Table 1). All the botanicals inhibited the mycelial growth of the pathogen. Aqueous extract of neem and Darek was found most effective amongst all the treatments with 38.26 and 38.10 per cent average mycelial inhibition respectivley, the two being at par. Aqueous extract of Kadu and Tulsi was found next in efficacy with mycelial inhibition of 34.56 and 33.82 per cent respectivley, the two being at par. Aqueous extract of hemp was found least effective with average mycelial inhibition of 16.01 per cent.

As the concentration increased from 10 to 50 per cent, there was corresponding increase in mycelial growth inhibition of the pathogen. At 50 per cent concentration, maximum mycelial inhibition of 58.88 per cent was observed in neem followed by 51.84 and 51.48 per cent in Darek and Tulsi respectively while least inhibition of 22.01 per cent was recorded in hemp.

Various workers have reported the efficacy of botanicals against *Pestalotia*/*Pestalotiopsis* infecting different hosts. Saha et al (2005) reported that aqueous extract of *A indica* resulted in 100 per cent mycelial inhibition of *Pestalotiopsis theae*. Ray et al (2016) evaluated five plant extracts against *P disseminata* and reported that *A indica* resulted in 95.56 per cent inhibition.

Botanicals are reported to have different constituent compounds which are reported to have growth inhibition properties against *Pestalotia* spp. The fungicidal properties of neem have been attributed to azadirachtin which belongs to C 25 terpenoides (Subramanian 1993).

The effectiveness of neem against plant pathogens has also been reported by Raheja and Thakore (2002). Among botanicals, neem-based products Neemnath 300, Neemnath 1500 and Godrej Achook were reported to cause 38.8, 44.4 and 33.3 per cent growth inhibition of *Pestalotiopsis heteronema* (Das and Pani 2002). Saju et al (2011) reported mycelial growth inhibition of *Pestalotiopsis* spp by aqueous extracts of *Artemisia vulgaris* and *Schima wallichi.* Effectiveness of eucalyptus leaf extract against *Pestalotia* spp is also reported by

Table 1. In vitro efficacy of botanicals against *Pestalotia* leaf spot pathogen (*Pestalotia longisetula*)

Treatment	Per cent inhibition in mycelial growth at different concentrations			
	10%	25%	50%	Mean
Neem	24.81 (29.84)	31.10 (33.88)	58.88 (50.12)	38.26 (37.94)
Tulsi	17.03 (24.22)	32.96 (35.02)	51.48 (45.83)	33.82 (35.02)
Banna	20.73 (27.02)	25.55 (30.33)	27.21 (31.42)	24.49 (29.59)
Hemp	10.42 (18.81)	15.66 (23.25)	22.01 (27.97)	16.01 (23.24)
Safeda	15.70 (23.27)	20.36 (26.80)	30.36 (33.41)	22.14 (27.83)
Aloe vera	12.58 (20.70)	37.44 (37.71)	41.77 (40.25)	30.24 (32.50)
Lemon grass	10.73 (19.08)	38.88 (38.56)	41.11 (39.86)	30.59 (32.88)
Artemisia	25.55 (30.22)	27.77 (31.74)	28.51 (32.25)	28.51 (31.40)
Kadu	23.33 (28.86)	34.44 (35.92)	45.93 (42.64)	34.56 (35.81)
Lantana	12.96 (21.04)	15.55 (23.21)	31.84 (34.31)	20.11 (26.19)
Menta	12.58 (20.70)	33.18 (35.16)	41.54 (40.12)	29.10 (31.99)
Ashwagandha	11.11 (19.45)	12.96 (21.04)	34.07 (35.68)	19.38 (25.39)
Darek	28.51 (32.22)	38.14 (38.11)	51.84 (46.04)	38.10 (37.79)
Cow urine	15.92 (23.37)	17.03 (24.35)	21.85 (27.85)	18.26 (25.19)
Mean	17.28 (24.20)	27.21 (31.07)	37.74 (37.69)	

Figures in parentheses are arc sine transformed values

$CD_{0.05}$

Treatments	1.91
Concentrations	0.88
Treatments x Concentrations	1.68

Wadud et al (2017). Seeds of *M azedarach* are reported to possess both fungistatic and fungicidal activities due to presence of organic molecules like vanillin, hydroxyl-3-methoxcinnamaldehyde and pinoresinol in them (Carpinella et al 1999, Mishra et al 2013).

In vitro evaluation of botanical field formulations (BFF-1 and BFf-2) and commercial neem formulation against *Pestalotia* leaf spot

In vitro evaluation of botanical field formulations BFF-1 (water-based), BFF-2 (cow urine-based) and commercial neem formulation (Neemazal) was also done. BFF-1 and BFF-2 at 5, 10 and 25 per cent and Neemazal at 1, 2 and 5 per cent were evaluated under in vitro conditions against *P longisetula* by poisoned food technique. The data on diametric growth of the test fungus were recorded and per cent mycelial growth inhibition was calculated (Table 2). All the treatments were effective in inhibiting the mycelial growth of the pathogen. Neemazal was found most effective with 92.59 and 83.66 per cent mycelial inhibition at 5 and 2 per cent concentration respectivley. BFF-2 was found next in efficacy with 68.96 per cent mycelial inhibition at 25 per cent concentration. Efficacy of each botanical formulation increased with increase in the concentration. Neemazal was found effective even at 1 per cent concentration with 51.09 per cent mycelial inhibition while BFF-2 resulted in 56.66 per cent mycelial inhibition at 10 per cent concentration.

Rai (1996) reported that among 17 plants extracts, *Eucalyptus globules* and *Catharanthus roseus* followed by *Ocimum sanctum* and *Azadirachta indica* proved highly effective against *Pestalotiopsis mangiferae* (leaf spot) in mango. Das and Pani (2002) reported the effectiveness of neem products viz Neemnath 1500 and Neemnath 300 against *P heteronema* with 44.4 and 38.8 per cent mycelial inhibition of the test fungus resepctively. Islam et al (2004) reported the effectiveness of *A indica* and *O sanctum* against *P palmarum*. Saha et al (2005) reported that aqueous extract of *A indica* and *Datura metel* proved highly effective against *P theae* and resulted in 100 per cent mycelial inhibition of test fungus. Saju et al (2011) in their work reported that combined aqueous extracts of *Artemisia vulgaris* and *Schima wallichi* proved effective against *Pestalotiopsis* spp.

Table 2. In vitro efficacy of botanical field formulations against *Pestalotia* leaf spot pathogen

Concentration (%)	Per cent inhibition in mycelial growth under different treatments		
	Botanical field formulation-1	Botanical field formulation-2	Neemazal
5	18.51 (25.46)	45.55 (42.43)	-
10	25.55 (30.34)	56.66 (48.86)	-
25	34.44 (35.92)	68.96 (56.13)	-
1	-	-	51.09 (45.61)
2	-	-	83.66 (66.25)
5	-	-	92.59 (74.24)
Mean	26.16 (30.57)	57.05 (49.14)	75.78 (62.03)

Figures in parentheses are arc sine transformed values

$CD_{0.05}$

Treatments	2.37
Concentrations	2.37
Treatments x Concentrations	4.10

Field efficacy of botanical field formulations

Two botanical field formulations (BFF-1 and BFF-2) and Neemazal were evaluated under field conditions. Both the botanical field formulations were evaluated at 10 per cent and commercial neem formulation (Neemazal) at 1 per cent concentration. Ten sprays of each treatment were given starting from the first week of July at 10 days interval. The data on per cent disease incidence, disease severity, yield (q/ha), plant height (cm) and number of runners per plant were recorded (Table 3).

All the treatments significantly reduced the incidence and severity of the leaf spot. Neemazal at 1 per cent and BFF-2 at 10 per cent were found most effective with disease incidence of 10.38 and 12.06 per cent and disease index of 4.24 and 6.54 per cent respectively. Between the two field formulations, BFF-2 (12.06%) was at par with BFF-1 (13.88%) for disease incidence but the disease index in BFF-2 (6.54%) was lower than BFF-1 (9.38%).

The yield recorded was 137.45, 141.34 and 138.00 q/ha and number of runners was 36.0, 37.6 and 35.8 in BFF-1, BFF-2 and Nemazal treatments respectively which were higher than control (53.68 q/ha and 13.8 respectively) but there were no differences among BFF-1, BFF-2 and Nemazal for these traits. Highest average plant height of 39.34 cm was recorded in the treatment BFF-2 followed by BFF-1 and

Table 3. Efficacy of botanical field formulations against *Pestalotia* leaf spot under field conditions

Treatment	Concentration (%)	Disease incidence (%)	Disease index (%)	Yield (q/ha)	Plant height (cm)	Number of runners/plant
Botanical field formulation-1	10	13.88 (21.81)	9.38 (17.78)	137.45	37.86	36.0
Botanical field formulation-2	10	12.06 (20.24)	6.54 (15.22)	141.34	39.34	37.6
Neemazal	1	10.38 (18.27)	4.24 (12.93)	138.00	36.72	35.8
Control		64.32 (53.33)	35.76 (12.34)	53.68	19.96	13.8
$CD_{0.05}$		2.13	2.38	4.76	2.43	2.57

Figure in parentheses are arc sine transformed values

Neemazal with plant height of 37.86 and 36.72 cm respectively, the latter two being at par.

Findings in the present study are corroborated by research findings of many other researchers. Pereira et al (2011) reported the efficacy of *Melaleuca* sp against *P longisetula* in strawberry under field conditions. Ara et al (2017) reported the efficacy of five botanicals (neem, Tulsi, bottlebrush, Arjun and *Aloe vera*) against *Pestalotiopsis* sp in strawberry under field conditions. Saju et al (2011) reported that combined application of aqueous extracts of *Artemisia vulgaris* and *Schima wallichi* were effective against *Pestalotiopsis* infecting large cardamom. Barman et al (2015) reported that aqueous extracts of garlic and Dhatura (1 mg/ml ethanol) were effective in reducing the leaf spots caused by *P theae* in tea under field conditions. Wadud et al (2017) reported the efficacy of eucalyptus leaf extract against leaf spot disease caused by *Pestalotia* spp in bay leaf (*Cinnamomum tamala*) under field conditions.

CONCLUSION

The present investigations were conducted to evolve an effective eco-friendly disease management strategy comprising botanicals to avoid the problem of pesticide residues in strawberry fruits. On the basis of the present study, it can be concluded that botanical formulation based on local bio-resources can provide effective alternative for the management of *Pestalotia* leaf spot in strawberry. Foliar sprays of cow urine-based field formulation at 10 days interval starting from first week of July were found effective and may be recommended for the management of disease under field conditions.

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