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Research Article

Effect of Different Botanical Extracts, Organic Compounds and Fungicide on the Management of Leaf Spot Disease of Strawberry (*Fragaria ananassa* Duch.) caused by *Pestalotia longisetula* under Field and In vitro Condition

Beautina Karki[1*], Hom Prasad Sitaula[2], Sandesh Bhandari[2], Pramod Gairhe[1], Hira Kaji Manandhar[1,2]

[1]Agriculture and Forestry University, Rampur, Nepal
[2]Nepal Plant Disease and Agro- Associates (NPDA), Nepal

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***Corresponding author**
Beautina Karki,
Agriculture and Forestry University, Rampur, Nepal
Email: beautykarki329@gmail.com

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Abstract

A field experiment was carried out using sweet sensation variety of strawberry to check the efficacy of different botanical extracts, organic compounds and chemical fungicide in order to manage leaf spot disease caused by *Pestalotia longisetula*. The study was done using ten treatments: ginger (T1), turmeric (T2), garlic (T3), ginger + turmeric + garlic (T4), cow urine (T5), cow urine + ginger + turmeric + garlic (T6), compost tea (T7), compost tea + ginger + turmeric + garlic (T8), SAAF (mancozeb 63% + carbendazim 12%) (T9) and control (T10), which were replicated thrice. The parameter observed during experiment were plant height, leaf number, disease leaves, stem lesions, yield, percent disease incidence, disease severity percent and percent disease control. At 75 days of transplantation, the highest plant height, leaf number and yield were observed in treatments compost tea (18.14 cm), cow urine + ginger + turmeric + garlic (24.60) and garlic (1391.67 gm/plot) respectively and lowest in treatment control (16.19 cm, 23.27 & 566.67 gm/plot). The number of diseased leaves and stem lesions were observed highest in treatment control (3.40 & 3.23), and lowest in treatments garlic (3.0) and cow urine + ginger + turmeric + garlic (1.83) respectively at 75 days after transplantation. Moreover, the highest percent disease incidence and disease severity percent were observed highest in treatment control (28.94% & 84.81%) and percent disease control in treatment garlic (42.36%) at 75 days of transplantation. In detach leaf experiment under in vitro condition, the lowest disease severity/intensity was observed in treatments garlic (40.74%) and turmeric (40.74%). Therefore, it is suggested to use garlic extracts as a measure to control *Pestalotia* leaf spot disease of strawberry.

Keywords: control; experiment; extract; treatment; yield

Introduction

Strawberry (*Fragaria ananassa* Duch.) is one of the most important soft fruit of the world. The strawberry plant is herbaceous, a perennial member of the rose family, Rosaceae. The much-shortened stem of the plant known as the crown, with leaves and axillary buds is borne in a restricted area of the apex. The natural succession of leaf development takes place in the strawberry plants where the oldest leaves become senescent and die and are continually replaced by new leaves developing from buds in the apical crown region (Mass *et al*., 1991). Botanically, strawberry is

not a berry but is an aggregate fruit. Amongst small fruits, it occupies an important place.

Due to advancements in plant breeding and biotechnology, a high degree of heterozygosity has been developed in Fragaria species enabling the development of strawberry cultivars adapting to a wild range of environmental conditions and resistant to diseases and pests. But in spite of the heterozygosity and advancement in technology, hundreds of fungi, many bacteria, and several viruses, MLOs and nematodes are reported in strawberries from different parts of the world (Mass, 1987). The other possible cause for the introduction of strawberry diseases is the increasing cultivation and importation of the planting material due to deficiency of certified planting material (Mouden *et al*., 2014).

Strawberry is infected by a number of disease-causing pathogens like fungi, bacteria, viruses, etc. Some of the common disease of strawberry includes red stele root rot, black root rot, leaf spot, fruit rot and blossom blight and leaf scorch (Carter & Henson, 2014). Leaf spots cause serious problems in the growth and yield of strawberry crops. Among these, leaf spots caused by *P. laurocerasi* and *P. longisetula* are important. The symptoms of *Pestalotiopsis spp*. appeared with the drying of the infected leaves which begins from the edges of the leaves. The appearance of the lesion begins from leaves and spread down the crown part. In severe conditions, drying of leaves takes place and the flower also turns black. Lesions are also seen in the stem and sometimes stem turns black (Dung *et al*., 2016). Ko *et al*. (2007) reported that small yellow to brown spots from a few millimeters to a few centimeters in diameter on leaves are the symptoms of *Pestalotia* spp. Later, the spots which are usually irregularly shaped turned white to grey and coalesced to form larger grey patches. The lesions had slightly dark raised margins. The characteristic symptoms of *Pestalotiopsis* spp. reported as small, 2-4 mm to 5-8 mm long, yellow, brown to black, irregular-shaped with dark brown to black margin, and brown-centered spot. The leaf spots usually turned grey with a black outline. Under optimum favorable conditions, the spots enlarge and increase in numbers until they merged to form leaf blight (Bhanwar *et al*., 2012). Pestalotiopsis leaf spot symptoms begin with small, yellow, brown or black spots which under optimum environmental expand and increase in their number and merge to form blight (Elliott, 2018).

For the management of foliar pathogens in strawberry, the use of botanical pesticides is one of the best alternatives (Stangarlin *et al*., 2015). Botanical pesticides have been popular these days because of its environment-friendly nature and have been reported to be effective against Pestalotia leaf spots in different crops (Rana *et al.,* 1999; Sindhan *et al*., 1929). The efficacy of *Melaleuca* spp. against *Pestalotiopsis longisetula* in strawberry was reported under field conditions (Pereira *et al*., 2011).

Materials and Methods

Experimental site and design

The field experiment was carried out in the farm of Berry World Private Limited during 2019-2020 at Dhapakhel, Lalitpur district. Planting materials were imported from India and planting technique used was raised bed with double row system. The experiment was conducted in randomized complete block design (RCBD) with ten treatments and three replications. The treatments used during experiments were mentioned in Table 1.

Table 1: List of treatments

Treatment number	Treatment detail
T1	Ginger (2%)
T2	Turmeric (2%)
T3	Garlic (2%)
T4	Ginger (1%) + Turmeric (1%) + Garlic (1%)
T5	Cow urine
T6	Cow urine + T4
T7	Compost tea (1:5)
T8	Compost tea + T4
T9	SAAF (mancozeb 63% + carbendazim 12%)
T10	Control

Variety used, spacing and plant population

Sweet sensation variety was used during the experiment, where row to row distance of 40 cm and plant to plant distance of 30 cm was maintained. There were 10 plots within each replication for 10 different treatments. 30 plants were planted in each of the plot and total of 900 plants for the all plots.

Isolation and identification of pathogen

The isolation of the associated pathogens was done from the diseased leaf samples which was collected from strawberry farms. The diseased leaves were taken for plating in petriplate by cutting into 2-3 mm size after surface sterilization with 1% sodium hypochlorite (NaOCl) solution. Some cut disease leaves were placed in 2% water agar media in petriplate (90 mm). These plates were incubated in incubator maintaining temperature of 25 ± 1^0C and observed after 48 hours for mycelia growth. After that mycelium of fungus was taken out using sterilized needle and observed in the compound microscope and identified the pathogen on the basis of the morphological characteristics. Thus, after the identification and confirmation the pathogen was transferred into PDA media to maintain its pure culture for further investigation.

Pathogenicity test

A ten-day old fully covered plate with mycelial mats of the test fungus (*Pestalotia longisetula*) was harvested by adding some sterilized distilled water with the help of spatula. The mixture was then strained through sterilized double layered muslin cloth to obtain conidial suspension. The supernatant

was then taken out and by adding sterilized distilled water. Spore count was done using haemocytometer and the concentration of the spores was adjusted to 2×10^7 spores/ml. Thus, prepared inoculum was used for pathogenicity test. Two methods were employed to prove pathogenicity.

a) Plant inoculation method: Healthy strawberry plants of Sweet Sensation variety were transplanted in plastic pots containing sterilized soil. The leaves were surface sterilized with 70% ethanol and then washed thoroughly with sterilized distilled water using hand atomizer. The spore suspension was sprayed on both surfaces of those leaves with the help of automizer. Control was maintained in which only sterilized distilled was sprayed in order to compare with inoculated plant. After inoculation, the plants were kept in a moist chamber made with the plastic sheet for 24 hrs. Thereafter, they were kept at room temperature. The plants were regularly observed for the appearance of any disease symptoms.

b) Detached leaf method: Healthy leaves were detached from healthy strawberry plants. The leaves were surface sterilized with 1% sodium hypochlorite (NaOCl) for one minute and then thoroughly washed with sterilized distilled water for two times. The leaves were transferred into Petri plate containing moistened sterilized blotting paper. Then the leaves were sprayed with the spore suspension with the help of hand automizer. The inoculated plates were then incubated in the incubator at 25±1°C. Control plate was also maintained for comparison where only sterilized distilled water was sprayed on the leaves. The leaves were observed daily for the appearance of symptoms of the disease.

Preparation and application of treatments

Rhizomes of fresh ginger and turmeric, and garlic cloves were washed with clean tap water, peeled and grinded with water in a blender. The ratio of plant materials and water used was 1:1 (weight: volume). The grinded materials were filtered through double-layered muslin cloth and considered the concentration of the extract as 100%. 200 ml of each botanical extract was mixed in 10 litres of water to make 2% concentration for field spray. Twenty-five days stored cow urine was used for the spray by diluting it with water at the ratio 1:5. Two litres cow urine was mixed with ten litres of water to make the final volume of twelve litres for field spray. For the combined treatment of cow urine with ginger (1%) + turmeric (1%) + garlic (1%) 100ml of each extract was mixed in ten litres of diluted cow urine with water (1:5). Compost tea was prepared as follows. Two kilograms of commercial compost was put into a muslin cloth keeping its mouth closed and was dipped into air tight container containing ten litres of water for seven days. It was regularly stirred in between twice a day. Thus, obtained completely black solution termed as 'compost tea' was used for drenching and foliar spray. For the combined treatment of compost tea with ginger (1%) + turmeric (1%) + garlic (1%) 100ml of each extract was mixed well in ten liters of prepared compost tea. Chemical fungicide SAAF was used at the recommended dose 2g per liters of water. Twenty grams of the fungicide was mixed in ten liters of water.

The treatments were applied by both spraying foliar parts and drenching seven days after transplantation. For drenching, 100 ml of each treatment preparation per plant was used. Control plots were sprayed and drenched with plain water. Total four sprays along with drenching were given at an interval of 15 days from 24th of September to 10th November 2019.

Observation

At 30 days after transplantation, first observation was taken. The following data were taken at every 15 days interval from tagged 10 plants per treatment in each replication.

a) Plant height: Plant height was calculated using measuring scale.

b) Leaf number, Disease leaves and Stem lesions: These parameters were counted by manual method through observation.

c) Yield: Yield was taken with multiple harvesting at an interval of seven days and weight was recorded using weighing machine.

d) Percent Disease Incidence (PDI) (%): It was calculated using formula as stated below:

$$\text{Percent disease incidence}(\%) = \frac{\text{Total no. of diseased leaves}}{\text{Total no. of leaves observed}} \times 100\%$$

e) Disease severity percent or Percent disease intensity (PDI) (%): It was calculated using following formula:

$$\text{Percent disease intensity (PDI)}(\%) = \frac{\text{sum of all ratings}}{\text{total no. of rating} \times \text{maximum disease grade}} \times 100\%$$

f) Percent Disease Control (PDC) (%): It was calculated using following formula:

$$\text{Per cent disease control}\ (\%) = \frac{\text{Percent disease incidence in control} - \text{Percent disease incidence in treatment}}{\text{Percent disease incidence in control}} \times 100\%$$

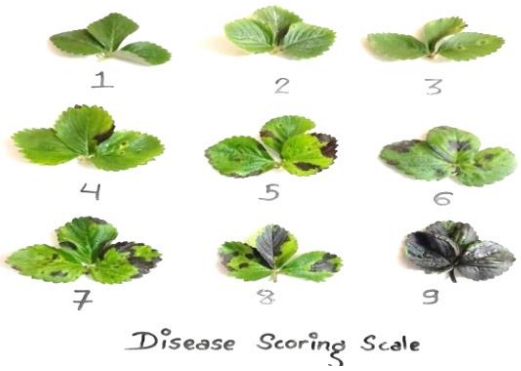

Fig. 1: Pictorial disease scoring scale

Table 2: Scoring scale (1-9) for *Pestalotia* leaf spot disease of Strawberry

Scale	Estimated plant part affected
1	No disease
2	1-10% area of leaf covered with spots
3	11-20% area of leaf covered with spots
4	21-30% area of leaf covered with spots
5	31-40% area of leaf covered with spots
6	41-50% area of leaf covered with spots
7	51-60% area of leaf covered with spots
8	61-70% area of leaf covered with spots
9	>71% area of leaf covered with spots

Source: (Manandhar *et al*., 2016)

g) Disease leaves scoring:

The disease scoring scale for Pestalotia leaf spot disease of Strawberry were shown in Table 2 and scoring disease samples in Fig. 1.

Data collection and analysis

The data recorded were tabulated in Microsoft Excel 2013 data worksheets. All the data were subjected to analyse by using the references of Gomez & Gomez (1984) and processing was done to fit into R-studio version 3.6.2 with agricolae 1.3-2 and ls means 2.30-0 for analysis of variance (ANOVA) and for data with significant difference, means compared by Duncan's multiple range test (DMRT).

Result and Discussion

Effect of treatments on plant height, leaves number and fruit yield

The plant height and leaves number go on increasing as days of transplanting (30, 45, 60 & 75 DAT) increased (Table 3). At 75 DAT, the plant height was found significantly highest in treatment compost tea (T7, 18.14 cm) which was statistically at par with treatments ginger (T1, 17.93 cm) and compost tea + ginger + turmeric + garlic (T8, 17.55 cm), whereas lowest plant height was found in treatment control (T10, 16.19 cm). No significant difference was observed in leaves number among different treatments at 75 DAT but comparatively highest leaves number was observed in treatment cow urine + ginger + turmeric + garlic (T6, 24.60) and lowest in treatment control (T10, 23.27). The average fruit yield per plot was observed significantly highest in treatment garlic (T3, 1391.67 gm/plot) which was statistically at par with treatments ginger (T1, 1088.33 gm/plot) and ginger + turmeric + garlic (T4, 1083.33 gm/plot) whereas lowest was observed in treatment control (T10, 566.67 gm/plot) respectively (Table 3).

Sayre (2003) reported that compost tea is one of the liquid manures. Scheurell (2003) posited that compost tea has been found to be a useful source of nutrients for both plant and microbial absorption. Compost tea helps in the mineralization of plant nutrients, fixation of nitrogen, and inhibit disease causing microorganism with the decomposition of toxic pesticides.

Effect of treatments on number of diseased leaves and stem lesions

The stem lesions and number of diseased leaves goes on increasing as days of transplanting (30, 45, 60 & 75 DAT) increased (Table 4). At 75 DAT, the number of disease leaves was found significantly highest in treatment control (T10, 3.40) whereas the lowest in treatment garlic (T3, 3.0), which was statistically at par with all other treatments except ginger + turmeric + garlic (T4) and compost tea (T7). Moreover, the number of stem lesions was found significantly higher in treatment control (T10, 3.23) whereas the lowest number of stem lesions was found in treatment cow urine + ginger + turmeric + garlic (T6, 1.83) which was statistically at par with all other treatments except compost tea (T7) and cow urine (T5) at 75 DAT.

For the management of foliar pathogens in strawberry, the use of botanical pesticides is one of the best alternatives (Stangarlin *et al*., 2015). These botanical extract have large amount of phytochemicals and have inhibitory effects on the growth of microorganisms (Malkhan *et al*., 2012). Garlic has been shown to have antibacterial, antifungal, and anti-oomycete action in vitro in several studies (Curtis *et al*., 2004).

Table 3: Effect of treatments on plant height, number of leaves and fruit yield of strawberry at Dhapakhel, Lalitpur, 2019

Treatments	Plant height (cm)				Leaves number				Average Yield/plot (gram)
	30 DAT	45 DAT	60 DAT	75 DAT	30 DAT	45 DAT	60 DAT	75 DAT	
T1	14.67^{abc}	17.12^{a}	17.78^{a}	17.93^{ab}	6.30^{e}	14.13^{d}	20.37	23.50	1088.33^{ab}
T2	14.12^{bc}	16.37^{ab}	17.13^{bc}	17.37^{bcd}	7.33^{ab}	14.77^{bcd}	20.83	23.70	1073.33a^{b}
T3	14.10b^{c}	15.85^{bc}	16.97^{bc}	17.25^{bcde}	6.97^{bcd}	15.70^{a}	21.50	24.30	1391.67^{a}
T4	15.430^{a}	16.43^{ab}	16.98^{bc}	17.26^{bcde}	7.17^{abc}	15.13^{ab}	21.07	23.83	1083.33^{ab}
T5	14.95^{ab}	16.32^{ab}	16.58^{cd}	16.76^{def}	7.50^{a}	15.10^{ab}	21.07	24.00	883.33^{bc}
T6	14.77^{ab}	15.95^{bc}	16.53^{cd}	16.95^{cde}	7.03^{bc}	15.47^{a}	21.47	24.60	766.67^{bc}
T7	15.50^{a}	17.10^{a}	17.87^{a}	18.14^{a}	7.20^{abc}	15.27^{ab}	21.37	24.20	700.00^{c}
T8	15.83^{a}	16.94^{a}	17.31^{ab}	17.55^{abc}	7.43^{ab}	15.03^{abc}	21.03	23.93	780.00^{bc}
T9	13.37^{c}	15.48^{c}	16.20d^{e}	16.52^{ef}	6.46^{de}	14.67^{bcd}	21.93	24.30	743.33b^{c}
T10	14.00^{bc}	15.28^{c}	15.84^{e}	16.19^{f}	6.83^{cd}	14.40^{cd}	20.40	23.27	566.67^{c}
LSD	1.20	0.73	0.59	0.66	0.45	0.62	1.02	0.87	318.27
GM	14.7	16.3	16.9	17.2	7.02	15	18	24	908
SEM	0.19	0.14	0.12	0.12	0.08	0.10	0.12	0.10	52.96
CV (%)	4.84	2.64	2.06	2.24	3.77	2.44	2.81	2.13	22.7

Note: DAT: Days After Transplantation, LSD: Least Significant Difference, GM: Grand Mean, SEM= Standard Error of Mean, CV: Coefficient of Variation; Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance.

Table 4: Effect of treatments on number of diseased leaves and stem lesions of strawberry at Dhapakhel, Lalitpur, 2019

Treatments	Disease Leaves Number				Stem Lesions			
	30 DAT	45 DAT	60 DAT	75 DAT	30 DAT	45 DAT	60 DAT	75 DAT
T1	1.80^{b}	2.43^{bc}	2.63^{bc}	3.20^{cd}	1.13^{cd}	1.56b^{cd}	1.77^{cd}	2.03^{cd}
T2	1.93^{b}	2.50^{bc}	2.63^{bc}	3.17^{cd}	0.80^{d}	1.27^{de}	1.63^{cd}	1.90^{cd}
T3	1.70^{b}	2.26^{c}	2.43^{c}	3.00^{d}	1.27^{bc}	1.63^{bc}	1.90^{bc}	1.93^{cd}
T4	2.10^{b}	2.73^{b}	2.83^{b}	3.43^{c}	1.30^{bc}	1.67^{bc}	1.83^{c}	2.03^{cd}
T5	2.17^{b}	2.63^{bc}	2.83^{b}	3.40^{cd}	1.23^{bc}	1.60^{bcd}	1.80^{c}	2.07^{c}
T6	2.10^{b}	2.67^{bc}	2.77^{bc}	3.33^{cd}	0.77^{d}	1.33^{cde}	1.63^{cd}	1.83^{d}
T7	3.07^{a}	3.73^{a}	4.20^{a}	5.33^{b}	1.60^{ab}	1.73^{b}	2.17^{b}	2.40^{b}
T8	2.03^{b}	2.77^{b}	2.93^{b}	3.33^{cd}	0.90^{cd}	1.13^{e}	1.47^{d}	1.80^{d}
T9	1.70^{b}	2.67^{bc}	2.83^{bc}	3.40^{cd}	1.03^{cd}	1.37^{cde}	1.73^{cd}	1.97^{cd}
T10	3.23^{a}	4.17^{a}	4.43^{a}	6.73^{a}	1.80^{a}	2.37^{a}	2.83^{a}	3.23^{a}
LSD	0.53	0.38	0.39	0.39	0.40	0.32	0.27	0.22
GM	2.18	2.86	3.05	3.83	1.18	1.57	1.88	2.12
SEM	0.10	0.11	0.13	0.22	0.06	0.06	0.07	0.08
CV (%)	14.2	7.85	7.53	5.96	19.5	12	8.47	6.22

Note: DAT: Days After Transplantation, LSD: Least Significant Difference, GM: Grand Mean, SEM= Standard Error of Mean, CV: Coefficient of Variation; Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance.

Table 5: Effect of treatments on percent disease incidence, percent disease intensity and percent disease control of *Pestalotia* leaf spot of strawberry

Treatments	Observations											
	30 DAT			45 DAT			60 DAT			75 DAT		
	PDI (1) (%)	PDI (2) (%)	PDC (%)	PDI (1) (%)	PDI (2) (%)	PDC (%)	PDI (1) (%)	PDI (2) (%)	PDC (%)	PDI (1) (%)	PDI (2) (%)	PDC (%)
T1	28.51^{b}	66.30^{bc}	13.12^{bcd}	17.25^{cd}	62.22^{cd}	21.86^{bc}	12.93^{bc}	60.37^{c}	26.93^{b}	13.63^{cd}	57.41^{b}	32.34^{b}
T2	24.43^{c}	64.81^{bc}	15.05^{bc}	16.97^{cd}	60.37^{de}	24.18^{ab}	12.67^{bc}	60.37^{c}	26.90^{b}	13.37^{cd}	57.78^{b}	31.88^{b}
T3	24.43^{b}	57.04^{d}	25.26 ^{a}	14.44^{d}	54.07^{e}	32.11^{a}	11.32^{c}	52.96^{d}	35.87^{a}	12.34^{d}	48.89^{c}	42.36^{a}
T4	29.35^{b}	65.56^{bc}	14.07^{bc}	18.08^{c}	61.11^{d}	23.25^{ab}	13.47^{bc}	59.26^{c}	28.25^{b}	14.40^{c}	55.56^{b}	34.50^{ab}
T5	28.97^{b}	63.33^{cd}	17.03^{ab}	17.44^{cd}	61.48^{d}	22.83^{bc}	13.46^{bc}	58.89^{c}	28.69^{b}	14.18^{cd}	54.81^{bc}	35.36^{ab}
T6	29.26^{b}	64.81^{c}	15.01^{bc}	17.26^{cd}	63.33^{cd}	20.44^{bc}	12.91^{bc}	61.11^{bc}	25.99^{bc}	13.57^{cd}	58.52^{b}	30.99^{b}
T7	42.76^{a}	74.07^{a}	2.92^{d}	24.47^{b}	75.19^{ab}	5.59^{d}	19.66^{a}	77.41^{a}	6.28^{d}	22.05^{b}	80.37^{a}	5.24^{c}
T8	27.29^{b}	72.59^{ab}	4.85^{cd}	18.4^{c}	68.89^{bc}	13.48^{cd}	13.95^{b}	66.67^{b}	19.29^{c}	13.93^{cd}	61.11^{b}	27.95^{b}
T9	26.24^{b}	66.30^{bc}	13.10^{bcd}	18.183^{c}	62.59^{cd}	21.41^{bc}	12.97^{bc}	61.11^{c}	26.02^{bc}	13.99^{cd}	58.52^{b}	31.03^{b}
T10	47.38^{a}	74.81^{a}	-	28.93^{a}	79.63^{a}	-	21.73^{a}	82.59^{a}	-	28.94^{a}	84.81^{a}	-
LSD	12.4	7.03	9.67	2.81	6.4	8.59	2.21	5.32	6.89	1.8	6.06	7.64
GM	31	67	13.4	19.1	64.9	20.6	14.5	64.1	24.9	16	61.8	30.2
SEM	1.48	1.14	1.48	0.79	1.45	1.56	0.62	1.67	1.62	0.95	2.09	2.00
CV (%)	23.9	6.12	41.7	8.56	5.75	24.1	8.87	4.84	16	6.52	5.72	14.6

Note: DAT: Days After Transplantation, PDI (1): Percent Disease Incidence, PDI (2): Percent Disease Intensity, PDC: Percent Disease Control, LSD: Least Significant Difference, GM: Grand Mean, SEM= Standard Error of Mean, CV: Coefficient of Variation; Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance.

Effect of treatments on percent disease incidence, percent disease intensity and percent disease control of Pestalotia leaf spot of Strawberry

The significant difference was observed among the treatments in percent disease incidence, percent disease intensity and percent disease control of *Pestalotia* leaf spot at 30, 45, 60 & 75 days after transplantation (DAT) respectively (Table 5). The percent disease incidence and percent disease intensity of *Pestalotia* leaf spot goes on decreasing, while that of percent disease control intensity of *Pestalotia* leaf spot goes on increasing as days of transplanting (30, 45, 60 & 75 DAT) increased. At 75 DAT, the highest percent disease incidence was observed in untreated control plots (T10, 28.94%) followed by treatment compost tea (T7, 22.05%) and the lowest in treatment garlic (T3, 12.34%). Similarly, the highest percent disease intensity was observed in control (T10, 84.81%) which was statistically at par with treatment compost tea (T7, 80.37%) and the lowest in treatment garlic (T3, 48.89%). Percent disease control was observed the highest in treatment garlic (T3, 42.36%) which was statistically at par with treatments cow urine (T5, 35.36%) and ginger + turmeric + garlic (T4, 34.5%), and the lowest in treatment compost tea (T7, 5.24%). The study conducted shows that botanical extracts and cow urine can cause reduction in the disease incidence percentage which corroborates with the evidences of Amin *et al.* (2013) where these compound increases the inhibition of mycelia growth and sclerotia formation.

Effect of treatments on total AUDPC, mean AUDPC and AUDPC per day

The significant difference was observed among the treatments for total AUDPC, mean AUDPC and AUDPC per day (Table 6). The highest total AUDPC was observed in control (T10, 3630.56), which was statistically at par with treatment compost tea (T7, 3447.22) and the lowest in treatment garlic (T3, 2400). Similarly, the highest mean AUDPC was observed in control (T10, 1210.19) which was statistically at par with treatment compost tea (T7, 1149.07) and lowest in treatment garlic (T3, 800). The highest AUDPC per day was observed in control (T10, 80.68), which was statistically at par with treatment compost tea (T7, 76.61) and lowest treatment garlic (T3, 53.33).

The observation of garlic extracts to have less AUDPC value corresponds with the findings of (Mahapatra & Srikanta, 2013). In two years, experiment conducted to manage Alternaria leaf blight of mustard using different botanical extracts, the minimum disease severity (AUDPC) was calculated in garlic bulb extract, which is par with neem leaf extract and highest disease severity (AUDPC) in the ginger rhizome.

Table 6: Effect of treatments on total AUDPC, mean AUDPC and AUDPC per day of *Pestalotia* leaf spot in strawberry, Dhapakhel, Lalitpur, 2019

Treatments	**AUDPC values**		
	Total	**Mean**	**Per day**
Ginger (T1)	2766.67^{bc}	922.22^{bc}	61.48^{bc}
Turmeric (T2)	2730.56^{c}	910.19^{c}	60.68^{c}
Garlic (T3)	2400.00^{d}	800.00^{d}	53.33^{d}
Ginger + Turmeric + Garlic (T4)	2713.89^{c}	904.63^{c}	60.31^{c}
Cow urine (T5)	2691.67^{c}	897.22^{c}	59.81^{c}
Cow urine + Ginger + Turmeric + Garlic (T6)	2791.67^{bc}	930.56^{bc}	62.04^{bc}
Compost tea (T7)	3447.22^{a}	1149.07^{a}	76.61^{a}
Compost tea + Ginger + Turmeric + Garlic (T8)	3036.11^{b}	1012.04^{b}	67.47^{b}
Saaf (T9)	2791.67^{bc}	930.56^{bc}	62.04^{bc}
Control (T10)	3630.56^{a}	1210.19^{a}	80.68^{a}
LSD	256	85.4	5.69
GM	2900	967	64.4
SEM	69.32	23.11	1.54
CV (%)	5.15	5.15	5.15

Note: AUDPC: Area Under the Disease Progress Curve, LSD: Least Significant Difference, GM: Grand Mean, SEM= Standard Error of Mean, CV: Coefficient of Variation; Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance.

Effect of treatments on disease severity/ intensity of detached leaf (Detached Leaf Experiment)

The significant difference was observed among the treatments in disease severity/intensity of detached leaf at 4, 6, 8, 10 & 12 days after inoculation (DAI) respectively (Table 7). The disease severity/intensity of detached leaf goes on increasing as days after inoculation (4, 6, 8, 10 & 12 DAI) increased. At 12th day, the highest disease severity was observed in control (T10, 100%) and the lowest disease severity was observed in treatments garlic (T3, 40.74%) and turmeric (T2, 40.74%) which were statistically at par with treatments ginger (T1, 48.15%) and ginger + turmeric + garlic (T4, 48.15%). The detached leaf method was shown in Fig. 2. Similar finding has been found in the management of blast disease of Rice on the use of a botanical extract where garlic extract has been found to be more effective as an alternative to conventional chemical fungicide (Netam *et al*., 2011).

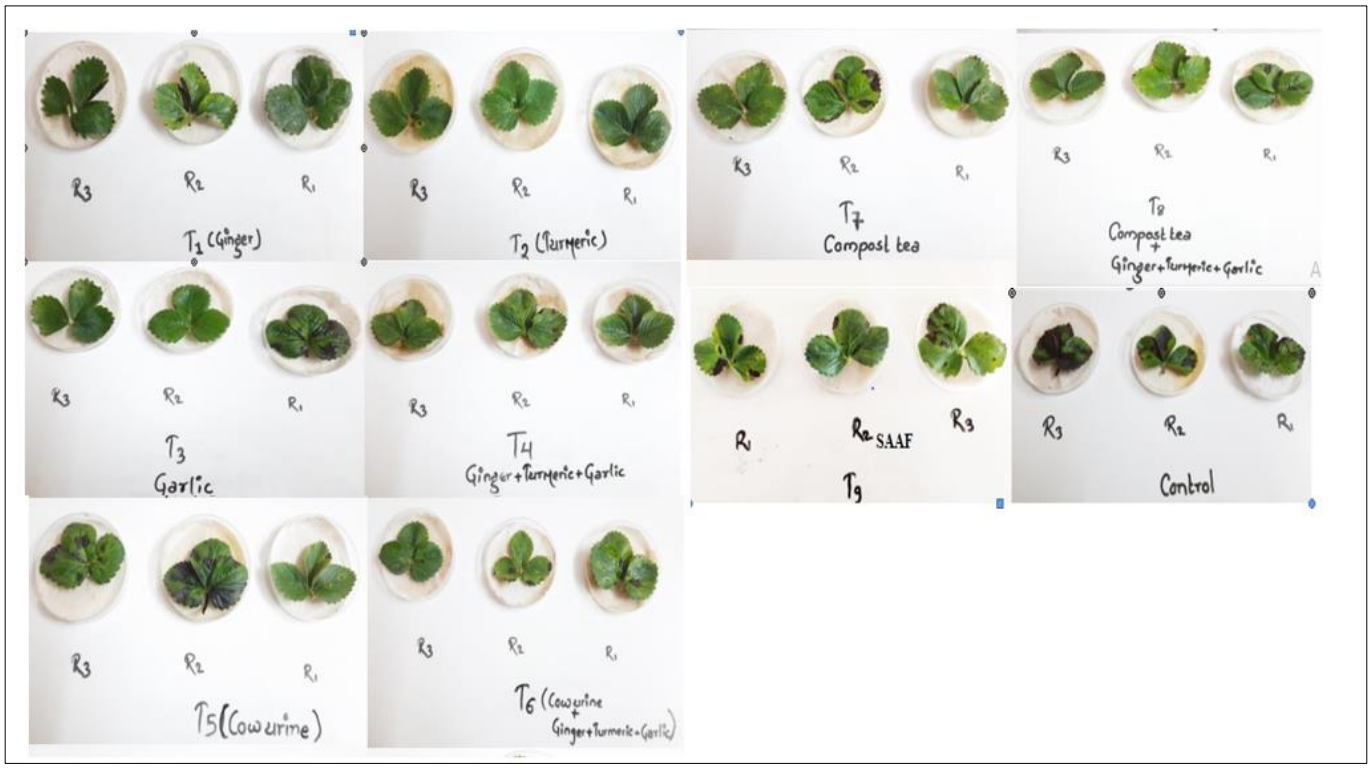

Fig. 2: Reaction of the inoculated detached leaves towards different treatments

Table 7: Effect of treatments on severity/intensity of *Pestalotia* leaf spot of strawberry on detached leaf under in vitro condition

Treatments	**Disease severity/intensity (%)**				
	4th day	**6th day**	**8th day**	**10th day**	**12th day**
Ginger (T1)	29.63^{d}	29.63^{cde}	29.63^{de}	37.04^{d}	48.15^{d}
Turmeric (T2)	25.93^{d}	25.93^{de}	29.63^{de}	29.63^{d}	40.74^{d}
Garlic (T3)	22.22^{d}	25.93^{de}	25.93^{e}	29.63^{d}	40.74^{d}
Ginger + Turmeric + Garlic (T4)	25.93^{d}	25.93^{e}	33.33^{de}	37.04^{d}	48.15^{d}
Cow urine (T5)	51.85^{b}	51.85^{b}	62.96^{b}	62.96^{bc}	74.07^{bc}
Cow urine + Ginger + Turmeric + Garlic (T6)	40.74^{c}	48.15^{b}	51.85^{bc}	55.56^{c}	66.67^{c}
Compost tea (T7)	40.74^{c}	44.44^{bc}	51.85^{bc}	66.67^{b}	77.78^{b}
Compost tea + Ginger + Turmeric + Garlic (T8)	29.63^{d}	40.74^{bd}	48.15^{c}	59.26^{bc}	70.37^{bc}
SAAF (T9)	22.22^{d}	25.93^{ef}	40.74^{cd}	55.56^{c}	66.67^{c}
Control (T10)	81.48^{a}	85.19^{a}	88.89^{a}	96.30^{a}	100.00^{a}
LSD	9.77	13.4	11.5	9.14	8.46
GM	37	40.4	46.3	53	63.3
SEM	3.34	3.51	3.54	3.72	3.42
CV (%)	15.5	19.5	14.5	10.1	7.85

Note: LSD: Least Significant Difference, GM: Grand Mean, SEM= Standard Error of Mean, CV: Coefficient of Variation; Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance.

Conclusion

Pestalotia leaf spot is one of the emerging and deleterious pathogens found to attack in the strawberry crops. Various botanicals extracts, organic compounds and chemical fungicide has been found effective to get remedy from the leaf spot disease of strawberry, among which garlic extracts is found to be better as compared to other treatments in controlling of the pathogens.

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Conflict of Interest

There is no any conflict of interest among the authors for the present study.

Authors' Contribution

Beautina Karki: Experimental design, conduction, data recording, analysis, interpretation and manuscript writing.

Hom Prasad Sitaula: Data recording, data analysis, interpretation and manuscript writing.

Sandesh Bhandari: Data recording, data interpretation and manuscript writing.

Pramod Gairhe: Data recording, data interpretation and manuscript writing.

Hira Kaji Manandhar: Supervision, suggestion and recommendation during experiment.

Final form of manuscript is seen and approved by all authors.

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