# MANAGEMENT OF POWDERY MILDEW OF STRAWBERRY (*FRAGARIAE* × *ANANNASA*) BY THE APPLICATION OF *ASCOPHYLLUM NODOSUM* EXTRACT

**by**

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## Abstract

Strawberry, an important fruit crop, is susceptible to a number of pathogens, which leads to a reduction in its productivity. *Ascophyllum nodosum* extract (ANE) is widely used as biostimulant. In this study, I evaluated the effect of ANE on powdery mildew of strawberry. The progression of disease was significantly reduced in ANE sprayed leaves in both detached as well as greenhouse-grown plants. In laboratory conditions, application of 0.2 % ANE showed maximum reduction in powdery mildew progression as compared to the control plants. ANE sprayed leaves showed higher phenolic and flavonoid content in response to powdery mildew infection. Furthermore, ANE application elicited defense response by inducing the activities of defense-related enzymes. Under field condition, ANE reduced the natural incidence as well as disease severity of powdery mildew. These results indicate that the ANE application increases the strawberry plant's active defense against powdery mildew by regulating the activities of defense-related enzymes.

## List of Abbreviations and Symbols used

| | |
|---|---|
| ANE | *Ascophyllum nodosum* extract |
| % | Percentage |
| PAL | Phenylalanine ammonia lyase |
| PPO | Polyphenol oxidase |
| PO | Peroxidase |
| FA | *Fragariae×anannasa* |
| RH | Relative humidity |
| cm | Centimetre |
| Mt | Million metric tonnes |
| Kt | Kilo tonnes |
| g | Gram |
| Kcal | Kilocalorie |
| mg | Milligram |
| µg | Microgram |
| α | Alpha |
| β | Beta |
| γ | Gamma |
| ξ | Delta |
| °C | Degree centigrade |
| *P. aphanis* | *Podosphera aphanis* |
| h | Hours (unit of time) |
| Spp. | Species |
| Syn. | Synonyms |
| NS | Nova Scotia |
| mL | Millilitre |
| $CH_3COOH$ | Acetic acid |

| | |
|---|---|
| $C_2H_5OH$ | Ethyl alcohol |
| DI | Disease intensity |
| LAI | Leaf area infection |
| rpm | Rotation per minute |
| M | Molar |
| mM | Millimolar |
| PMSF | Phenylmethylsulphonylfluoride |
| PVP | Polyvinylpyrrolidone |
| g/l | Gram per litre |
| w/v | Weight by volume |
| nm | Nanometre |
| $H_2O_2$ | Hydrogen per oxide |
| N | Normality |
| $Na_2CO_3$ | Sodium carbonate |
| UV | Ultraviolet |
| DW | Distilled water |
| $AlCl_3$ | Aluminium chloride |
| $NaNO_2$ | Sodium nitrite |
| NaOH | Sodium hydroxide |
| QE | Quercetin equivalents |
| GAE | Gallic acid equivalents |
| FW | Fresh weight |
| HCl | Hydrochloric acid |
| $CO_2$ | Carbon di oxide |
| DI | Disease incidence |
| DS | Disease severity |
| RCBD | Randomized complete block design |
| CRD | Complete randomized design |

| | |
|---|---|
| ANOVA | One-way variance |
| CI | Confidence interval |
| SAS | Statistical analysis system |
| FA | Foliar application |
| Fig. | Figure |
| Hpi | Hour post inoculation |
| > | Greater than |
| ≤ | Less than or equal to |
| C | Control |
| T1 | Treatment one (0.1 %) |
| T2 | Treatment two (0.2 %) |
| T3 | Treatment three (0.3 %) |
| ± | Both plus and minus operation |
| SE | Standard error |
| n | Number |
| P | Probability |
| dpi | Days post-inoculation |
| sq.mm | Square millimetre |
| PAMPs | Pathogen associated molecular patterns |
| PRRs | Pattern recognition receptors |
| BTH | Benzothiadiazole |

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# 1.0 INTRODUCTION

Strawberry (*Fragaria* x *anannasa*) is an important small fruit cultivated globally. In Canada, strawberry is a significant berry crop with production totaling 19,372 metric tonnes and a market value of $ 82.5 million (Statistics Canada 2015). Global effects like desertification, soil salinization, atmospheric $CO_2$ enrichment and nutrient imbalances (including mineral toxicity and deficiency) cause dramatic changes in the environment of agricultural lands (dos Reis et al., 2012). These changing environmental conditions have the potential to increase plant susceptibility to pathogens, as most of the plant diseases are products of interaction between susceptible host plants, virulent pathogens, and the environment (Elad and Pertot, 2014; West et al., 2012). Strawberry yield and fruit quality are largely affected by different biotic and abiotic stresses. Among biotic factors, fungal diseases are major limiting factors that cause significant losses in strawberry productivity (Elmhirst 2005; Nezhadahmadi et al. 2015).

Powdery mildew is caused by a bio-trophic fungal pathogen *Podosphaera aphanis* (syn. *Sphaerotheca macularis*) (Kiss 2003) and is a major limiting factor in strawberry production worldwide (Maas 1998). The asexual stage of this pathogen on strawberries was first reported in 1854 in the United Kingdom (Corke and Jordan 1978). Powdery mildew infects all parts of the strawberry plant including leaf, fruit, peduncle, stem, and runners. Initial symptoms of powdery mildew appear as a white powdery formation on the leaf surface followed by an upward curling of leaves, white mycelia on the upper surface of the leaf and lower leaves turning

reddish in color (De Cal et al. 2008). *P. aphanis* causes severe damage to the leaves and can reduce photosynthetic activity due to dense mycelial growth causing necrosis, ultimately resulting in defoliation and yield loss (Karajeh et al. 2012). Many commercially available strawberry cultivars are highly susceptible to powdery mildew, and it is generally suppressed by the application of fungicides such as captan and benomyl (Wedge et al. 2007). There are only a few moderately resistant varieties available, but these varieties cannot eliminate the requirement of fungicide application for disease suppression. For the management of plant diseases, current agricultural practices involve excessive use of chemical-based pesticides (McCallan 1949). These chemicals have profound harmful consequences on the environment and human health. Therefore, alternate eco-friendly approaches for the management of powdery mildew need to be explored.

Seaweeds are an important component of marine coastal ecosystems (Shukla et al. 2016). Seaweeds are a rich source of macro- and micronutrients, amino acids, vitamins, and growth substances that affect cellular metabolism and enhance plant growth and crop yield (Khan et al. 2009). The most commonly used seaweed as a plant bio-stimulant is *A. nodosum* (rockweed), a brown alga, widely distributed in the North Atlantic Ocean. *Ascophyllum nodosum* is rich in polysaccharides (alginic acid, fucoidan and laminarin), minerals and vitamins. It is also rich in bioactive compounds such as polyphenols, lipids and proteins (Holdt and Kraan 2011). The extract from *A. nodosum* has great potential as a disease protectant by enhancing the activities of various defense-related enzymes and

aiding plants to reduce the disease (Righini et al. 2018; Jayaraman et al. 2011; Hernandez-Herrera et al., 2014).

### 1.1 Hypothesis:

The hypothesis of this research was ANE will enhance plant defense against powdery mildew of strawberry.

### 1.2 Objective:

The goal of this research was to evaluate the efficacy of the extract of *Ascophyllum nodosum* in reducing powdery mildew disease in strawberry, both in the greenhouse as well as under field conditions.

# 2.0 REVIEW OF LITERATURE

## 2.1. Strawberry:

Strawberry is very popular and widely consumed due to their characteristic aroma, bright red color, juicy texture, and sweetness (Khoshnevisan et al. 2013, 2014). The strawberry belongs to the *Rosaceae* family of *fragariae* genus with 23 species. The cultivated variety of strawberry (*Fragariae anannasa*) originates from a cross between *Fragariae chinoensis* and *Fragariae virginiana.* Strawberry is a perennial dicotyledonous herbaceous plant that produces trifoliate leaves densely covered with trichomes, and stolons which are used for propagation (Figure 1). Strawberry plants grow best in deep, well-drained sandy-loam soils with a slightly acidic pH (5.5-6.5) but also grow well in a wide variety of soil types ranging from sandy to clay loam. Globally, strawberry plants grow either on matted rows or in a plastic culture system. In matted rows, the stolon is treated as the primary yield component and allows both mother and daughter plants to grow together, while in the plastic culture system, the crown is treated as the primary yield component and stolon is removed from mother plants (Hancock et al. 2008). Strawberry plants have a shallow root system, therefore requiring adequate spacing between rows (46-76 cm) for good quantity and quality of the fruit, which also reduces the incidence and severity of numerous diseases (Elmhirst 2005; Hancock et al. 2008).

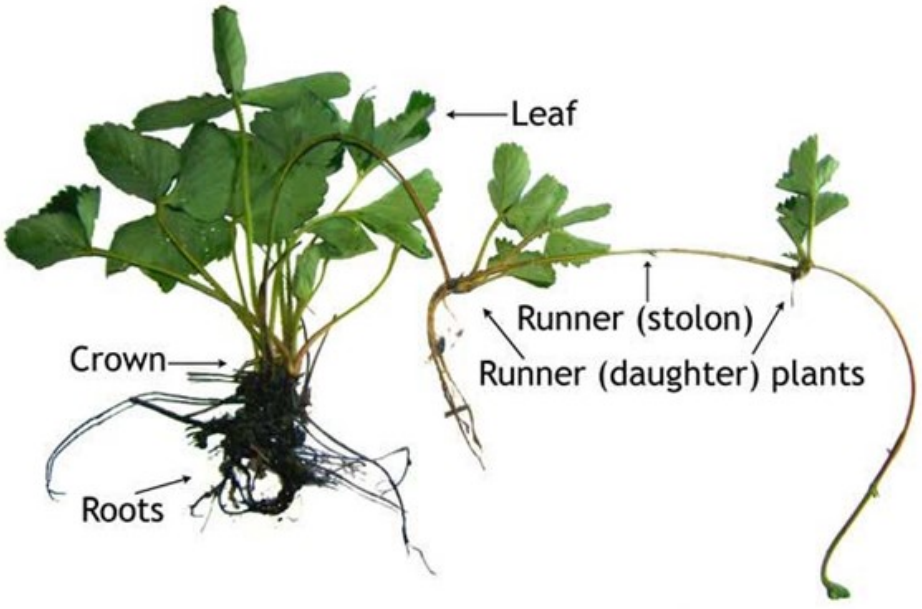


**Figure 1. Picture of a strawberry plant with major anatomical features including roots, crown, leaf arrangement and daughter plants (Adapted from www.shutterstock.com).**

Strawberry is one of the major fruit crops in several countries and contributes greatly to their economies. Globally, Asia leads in production of strawberry (Figure 2). China and the USA are the main strawberry producers with about 3.8 and 1.4 Million metric tonnes, followed by Mexico, Turkey, and Spain with about 468, 415, and 366 kt in 2016.

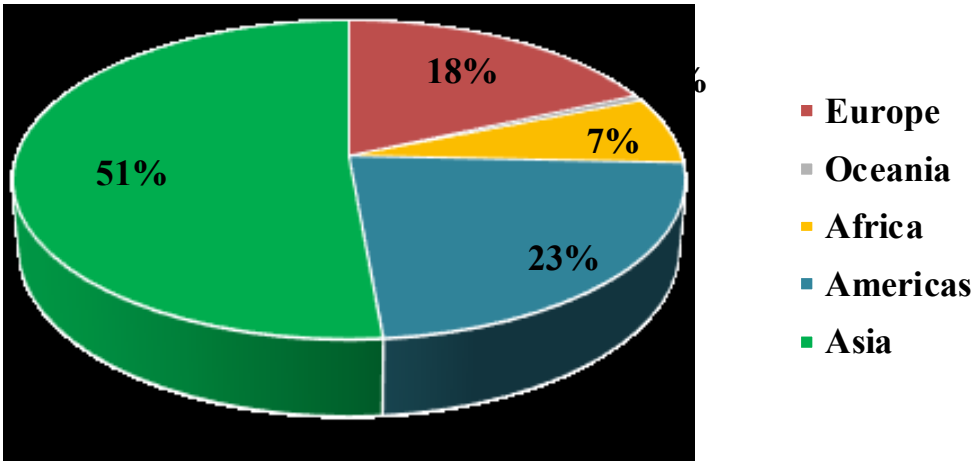


**Figure 2. Percentage distribution of strawberry production worldwide (Adapted from Rubinstein 2015).**

Economically, strawberry is a very important horticultural crop grown worldwide. In Canada, strawberry is one of the most important fruit crops grown in almost all the provinces including Quebec (36%), Ontario (32%), British Colombia (15%), and Nova Scotia (7.9%) followed by New Brunswick (2.8%), Manitoba (2.7%), Alberta (1%) Prince Edward Island (0.9%), Newfoundland and Labrador (0.9%), and Saskatchewan (0.5%) (Figure 3) (Elmhirst 2005). Total production of strawberry in Canada is 19,372 metric tonnes in 3,279 hectares of land with a market value of $82.5 million (Statistic Canada 2015).

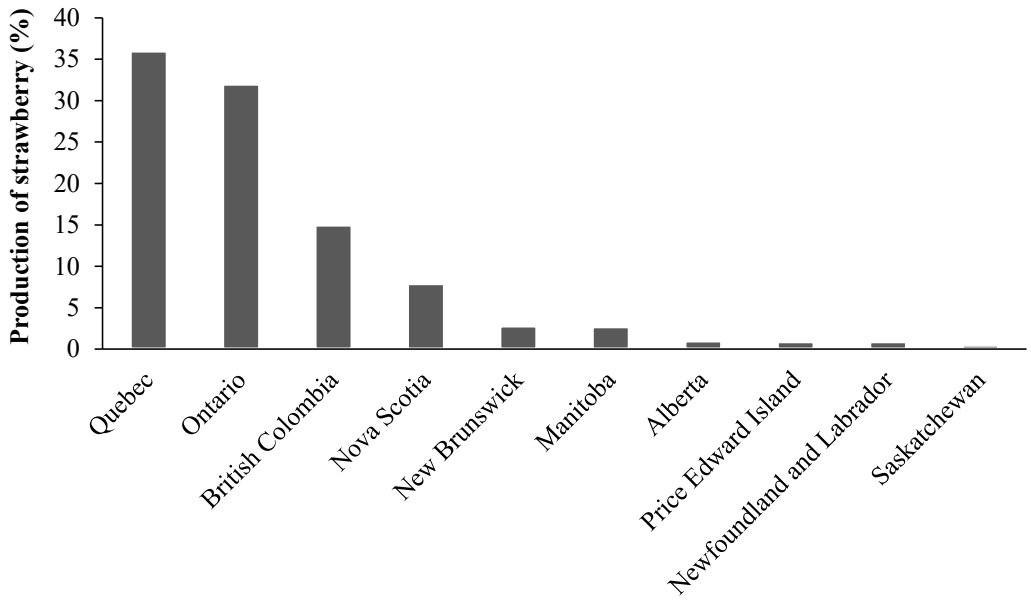


**Figure 3. Strawberry crop production in Canada (adapted from Statistic Canada 2015)**

Strawberry are classified into three types: June bearers (short day), day neutral and ever bearers (long day). The June bearer is the most commonly planted strawberry. They are vegetatively grown and produce numerous runners during the summer and fall seasons when the nights are comparatively longer and are often planted in a matted row system. Day neutral variety produces flowers and fruits during the summer season. This variety is not influenced by the day length, but they do not produce flowers and fruits during hot weather. The productivity and fruit quality of this variety is much better than June bearers. The ever-bearing cultivar of strawberry produces few flowers and fruit in late summer and late spring. This variety produces some runners.

## 2.2. Health benefits of strawberry:

Strawberries are a beneficial fruit for human health due to their high content of phyto-chemicals, vitamins, fatty acids and minerals. It is considered as a functional fruit due to its various array of nutritional composition and fibers (Table 1) (Afrin et al. 2016). The strawberry is an appropriate source of bioactive compounds due to its high content of vitamin C, folate, and phenolic constituents (Giampieri et al. 2012). It is economically and commercially important, and widely consumed in fresh or processed forms (Proteggente et al. 2002). Strawberry also contributes to the regulation of blood sugar levels due to high fiber and fructose content. It is also a rich source of essential fatty acids (Proteggente et al. 2002).

**Table 1. Nutritional composition of strawberry** (Giampieri et al. 2012).

| **Nutrients** | **per 100 g of strawberry** |
|---|---|
| Water | 90.92 g |
| Energy | 32 kcal |
| Protein | 0.67 g |
| Total lipid | 0.3 g |
| Carbohydrate | 7.68 g |
| Dietary fiber | 2 g |
| Sucrose | 0.47 g |
| Fructose | 2.44 g |
| Glucose | 1.99 g |
| Ca | 16 mg |

| | |
|---|---|
| Mg | 13 mg |
| Iron | 0.41 mg |
| Sodium | 1 mg |
| Potassium | 153 g |
| Phosphorus | 24 mg |
| Vitamin C | 58.8 mg |
| Thiamin | 0.024 g |
| Riboflavin | 0.022 mg |
| Niacin | 0.386 mg |
| Vitamin B6 | 0.047 mg |
| Folate | 24 µg |
| Vitamin A | 1 µg |
| Lutein, Zeaxathin | 26 µg |
| Vitamin E | 0.39 mg |
| Vitamin K, phylloquinone | 2.2 µg |

## 2.3. Fruit quality of strawberry:

Strawberry fruit is one of the most appreciated berry fruits for its high nutritional value. It is a very delicate fruit and prone to several diseases. Numerous biotic and abiotic stresses affect strawberry yield and fruit quality. Among biotic factors, bacterial and fungal diseases cause significant damage and the disease issue is increasing exponentially, making disease management challenging (Elmhirst, 2005; Nezhadahmadi et al. 2015; Kim et al. 2016). Among all diseases, powdery mildew is one of the most serious problems in strawberry growing areas. This

disease can cause yield loss up to 80 % of total production by making the fruit unmarketable.

## 2.4. Major strawberry diseases:

Several leaf and fruit diseases of strawberry develops during summer and fall season in all strawberry growing areas. These diseases slowly cause devastating damage to this fruit (Paulus 1990). Strawberry quality and quantity are affected by several diseases.

Some of the most important fungal diseases in strawberry growing areas are listed in Table 2 with their causative agent and affected plant parts (Hancock et.al., 2008).

**Table 2. Major fungal diseases of strawberry with causal organisms**

| **Disease name** | **Pathogen name** | **Affected plant part** |
|---|---|---|
| Gray mould | *Botrytis cinereal* | Flower blight, rotting of green and ripe fruit |
| Anthracnose fruit rot | *Colletotrichum acutatum* | Foliage, runners, crowns and fruit |
| Red core | *Phytophthora fragariae* | Roots (Root tips and lateral roots) |

| | | |
|---|---|---|
| Verticillium wilt | *Verticillium albo-atrum* | Leaf drooping, wilting, reddish yellow or brown at margins and between the veins |
| Leather rot | *Phytophthora cactorum* | Flower buds, green or ripe fruit |
| Powdery mildew | *Podosphera aphanis* (*Syn.Sphaerotheca macularis*) | Fruit, runner, leaf and stem |

## 2.5. Powdery mildew:

Fungi causing powdery mildew are one of the most conspicuous groups of plant pathogens with more than 500 species that affect more than 1500 plant genera. Several major crops are the soft target of this disease (Kiss 2003). Powdery mildew reduces crop yield ranging from 30 % - 100 % depending on the crop species (Peetz et al. 2009). Powdery mildew is one of the most destructive diseases of strawberry plants worldwide (Amsalem et al. 2006). Powdery mildew is caused by a bio-trophic fungal pathogen *Podosphaera aphanis* (Syn. *Sphaerotheca macularis)* (Kiss 2003) and is a serious problem in strawberry growing areas worldwide. Powdery mildew affects all parts of the strawberry plant except the root. Yield and productivity are directly affected by Powdery mildew as the disease causes the fruit to become unmarketable. Heavy mycelial growth on foliage reduces the photosynthetic activity (Amsalem et al. 2006) and ultimately results in defoliation. The asexual stage of this pathogen on strawberries was first reported in 1854 in the United Kingdom (Corke and Jordan 1978). The fruting body of the

sexual stage of *P. aphanis* known as cleistothecia have also been reported but it is not common (Corke and Jordan 1978). Current management of Powdery mildew on strawberry depends on disease free transplantation and through multiple chemical fungicide applications (Xiao et al. 2001).

### 2.5.1. Taxonomy:

Powdery mildew is caused by pathogen *Podosphera aphanis* (Syn. *Spherotheca macularis*), belongs to phylum Ascomycota with Leotiomycetes class and Erysiphaceae family.

### 2.5.2. Optimal conditions for disease development:

Several studies showed that the disease incidence of powdery mildew fungi is positively correlated with the conidial presence in air (Blanco et al. 2004). The optimum temperature needed for the germination and infection of spores ranges from 15-25 °C with high relative humidity 75-98 % (Karajeh et al. 2012). Peries (1962) showed that the conidial germination can occur from a minimum temperature of 2 °C to the maximum of 25 °C, but the germination rate slows down between the temperature ranges 5-13 °C. Therefore, the optimum temperature for infection, growth and conidial production is between 18-25 °C with high relative humidity 95 % (Peries 1962; Miller 2003; Peetz 2008; Amsalem et al. 2006).

### 2.5.3. Life cycle:

The fungi *P. aphanis* is found in all strawberry growing areas. Under the favorable condition (18-25°C) with high relative humidity (95%) this biotrophic fungal pathogen produces conidia on specialised conidiophores (Peries 1962; Miller

2003; Peetz 2008). Conidia is dispersed through wind, and when they land on a susceptible host, they initiate the infection by producing mycelial colonies.

*P. aphanis* also produces sexual spores known as ascospores enclosed in an ascocarp (fruiting body). Each ascocarp contains a single ascus with eight ascospores. Ascocarp is identified as typical black dots on the mycelial surface. The sexual stage is known as clestothesia (a closed structure with appendages) (Figure 4).

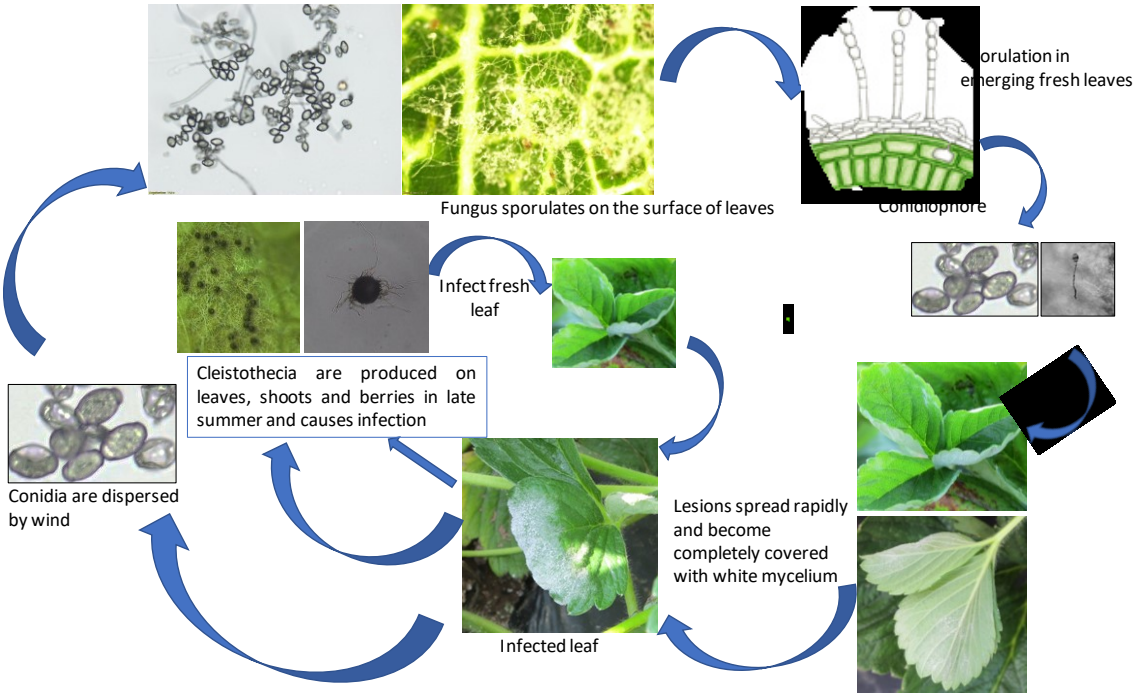


**Figure 4. Life cycle of *P. aphanis* (*Syn. S. macularis*)**

### 2.5.4 Disease cycle:

When the conidia of powdery mildew lands on the host plant surface, the chain of complex processes starts. In the presence of favorable environmental conditions

(18-25°C) with high relative humidity (95%), the susceptible host gets powdery mildew infection. Initially the conidia lands on the surface, germinates, and starts penetrating the host cuticle and cell wall. The penetration occurs due to the development of appressorium (a special penetrating organ which forms as a swelling from the germ tube tip).

#### 2.5.4.1 Infection:

Powdery mildew is caused by an obligate fungal pathogen and requires a living host for its development. Therefore, this pathogen cannot grow on artificial media. In optimal conditions (with high relative humidity and temperature), the conidia of powdery mildew germinate within four hours of landing and the appressorium formation occurs after 12 hours (Corke and Jordan 1978). After successful penetration, *P. aphanis* derives their nutrition from the host plant by developing an artificial feeding organ known as haustorium. This fungus produces white powdery ascospores or conidia (asexual stage of spores), which can disperse through air and can also be transmitted through infected planting material. (Tenhovirta 2012; Amsalem et al. 2006).

#### 2.5.4.2 Symptoms:

The powdery mildew fungi affect all parts of strawberry plants: leaves, petioles, fruits and runners. Initially, the pathogen develops as white powdery mycelial growth on the lower leaf surface and as the disease progresses, it appears on the upper surface as well. In severe conditions, leaves start curling and discoloration occurs (Palmer 2007). The mycelial growth causes severe damage to the foliage

resulted in necrosis and defoliation and ultimately reduces the photosynthetic activity (Karajeh et al. 2012).

## 2.6 Management practices:

Major management practices adopted for the control of plant diseases are crop rotation, fungicide application, and use of resistant crop varieties (Kiss 2003; Karajeh et al. 2012).

Crop rotation is an effective cultural method for reducing diseases in the field (Reis et al. 2011). Like other control methods, crop rotation needs better understanding of the biology of the pathogen for best results. Some pathogens can survive from year to year, therefore growing the same crop can build up a high pathogen population, but by growing a non-host crop within the rotation, the pathogen population can be reduced (Singh and Chawla, 2012). The rotation of crops within the same family or with similar pathogenic tendencies can lead to the development of resting structures that can survive in soil for a long period of time (Peters et al. 2003; Peng et al. 2015). Earlier studies have reported that three to five years of crop rotation with completely non-host crops significantly reduce the incidence of disease on different crops (Morrall and Dueck, 1982).

For the management of powdery mildew, prevention is the best way to minimize the disease either by using resistant cultivars or by following good cultural practices. There are a wide range of fungicides available (mostly copper based) for the management of powdery mildew disease of strawberry that can be effective in protecting production and yield after multiple applications (Karajeh et al. 2012).

The application of these fungicides has numerous adverse effects on the environment and can ultimately impact human health by leaving the toxic residues on the harvested crop (Nicolopoulou-Stamati et al. 2016; Mie et al. 2017). Fungicides are also toxic to bees (Kiss 2003; Verweij et al. 2016), and aquatic life through water body contamination (McMahon et al. 2012; Jayaraman et al. 2011). The commercial fungicides that are used for powdery mildew management are captan, Tetraconazole, benomyl. Similarly, the fertilizer application, soil health, site selection, weed and pest management are also important tools for plant disease management. Knowledge about the types of plant diseases and their common host is an important step for plant disease management (Krupinsky et al. 2002).

Other than biocontrol agents, the application of plant products in plant disease management appears a logical and effective method (Prithiviraj and Singh 1995). The best way for the management of any plant disease to minimize incidence and severity is through identifying the signs and symptoms, pathogen behavior and favorable environmental conditions for pathogen growth. To minimize the use of pesticides, other sustainable, environmentally friendly disease management practices must be explored.

## 2.7 Seaweeds in Agriculture:

Due to the harmful effect of synthetic chemicals on the environment and human health, there is a considerable interest in developing sustainable approaches to manage plant diseases as an alternative to synthetic pesticides (Crouch and Van Staden 1993). In the last few decades, the use of seaweed products in the

agricultural and horticultural industries has extensively increased. Seaweeds are important constituents of the marine coastal ecosystem with macroscopic, multicellular structures (Shukla et al. 2016). In ancient times, seaweeds were used in crop production systems for plant growth and yield enhancement (Alam et al. 2013). The existing literature shows that seaweeds induce several disease resistant factors and reduces the rate of disease severity in crop plants (Jayaraj et al. 2008; Alam et al. 2013). Seaweeds are a rich source of macro and micronutrients, amino acids, vitamins, and plant growth regulators that affect cellular metabolism and can enhance plant growth and crop yield. Seaweeds are also rich in polysaccharides (fucoidan, laminaran and alginic acid) and bioactive compounds such as lipids, proteins and polyphenols (Holdt and Kraan 2011). Seaweeds are generally used in agriculture either as spray or as soil amendments to improve the plant health or crop yield (Chapman 2012). Based on their pigmentation, seaweeds are broadly classified into three types: *Phaeophyta* (Brown algae), *Rhodophyta* (Red algae) and *Chlorophyta* (Green algae) (Khan et al. 2009). Among these, *Phaeophyta* (Brown seaweed) is the second richest group comprising about 2000 species (Khan et al. 2009; Alam et al. 2013).

Macroalgae, such as *Ascophyllum nodosum, Ecklonia maxima, Fucus spp*., *Laminaria spp*., *Sargassum spp., and Turbinaria spp*., have been most researched as a commercial source for the large-scale production of bio-effectors and of biostimulants for plant growth and health (Van Oosten et al. 2017). Throughout the world, nearly 47 companies are currently involved in the preparation of 89

commercial extracts from *A. nodosum* for agricultural applications (Van Oosten et al. 2017). *Ascophyllum nodosum* (ANE) (rockweed) is widely distributed through the coastal areas of the North Atlantic Ocean. *A. nodosum* is renowned for its richness of polysaccharides, minerals and vitamins. It is also rich in multiple bioactive compounds such as polyphenols, lipids and proteins (Craigie 2011; Holdt and Kraan 2011). ANE is also a rich source of phenolic compounds and secondary metabolites which are synthesized during any biotic or abiotic stress. Phenolic compounds play an important role in anti-oxidant activity and protect cellular components from any damage (Battacharyya et al. 2015). *A. nodosum* has various beneficial properties in agriculture, including early seed germination and establishment, higher productivity and enhanced tolerance to various biotic and abiotic stresses (Shukla et al. 2018; Khan et al. 2009).

*A. nodosum* extract is the most extensively studied for its plant growth-promotion activity (Khan et al. 2009). ANE promotes root and shoot growth of Arabidopsis by regulating phyto-hormone metabolism (Rayorath et al. 2008; Wally et al. 2013). Global transcriptomics of Arabidopsis in the presence of ANE showed that it regulates many genes involved in plant growth and development (Goñi et al. 2016).

*A*. *nodosum* is not only involved in improving growth and crop yield, but there are several reports which suggested that it also acts as a potential elicitor and possesses disease suppressive activities (Khan et al. 2009). *A. nodosum* reduces crop damage caused by different plant pathogens by stimulating systemic

resistance (Ali et al. 2016). Previously published reports have revealed that the disease resistance in plants might be mediated by the expression of several defense genes, higher production of defensive enzymes, PR proteins and accumulation of secondary metabolites (Ali et al. 2016; Subramanian et al. 2011; Jayaraman et al. 2011). The root drench and foliar application of ANE improves growth rates, reduces incidence of pests and insects, enhances crop yield and improves overall crop quality (Khan et al. 2009).

### 2.7.1 *Ascophyllum nodosum* extract (ANE) and plant growth:

Considering concerns regarding the excessive use of pesticides in horticultural crops, there is a need to develop an alternative strategy for sustainable disease management in agricultural system (Di Stasio et al. 2018). The use of biostimulants from marine sources offers an alternative strategy for crop improvement (Bulgari et al. 2015). ANE stimulates plant growth and improves crop yield as it constitutes micro and macro nutrients, amino acids, plant growth regulators such as cytokinins, absisic acid and auxins (Table 3), (Shukla et al., 2019). Currently, leading biostimulant companies such as Acadian Seaplants Limited are exploring extracts from *A. nodosum* as a source of bioactive compounds: ANE is biodegradable, economic (low cost), non-toxic to the environment and humans, animals and other creatures (Rathore et al. 2009; Dhargalkar and Pereira 2005).

**Table 3. Chemical composition of *Ascophyllum nodosum* Extract (Adapted from Acadian Seaplants, Hurtado et al. 2008)**

| | |
|---|---|
| **Physical data** | |
| Appearance | Brownish-black crystals |
| Odor | Marine |
| Solubility in water | 100% |
| pH | 10.0-10.5 |
| **Typical analysis** | |
| Maximum moisture | 6.5% |
| Organic matter | 45-55% |
| Ash (Minerals) | 45-55% |
| Total Nitrogen (N) | 0.8-1.5% |
| Phosphoric acid ($P_2O_5$) | 1–2% |
| Soluble potash ($K_2O$) | 17–22% |
| Sulfur (S) | 1–2% |
| Magnesium (Mg) | 0.2–0.5% |
| Calcium (Ca) | 0.3–0.6% |
| Sodium (Na) | 3–5% |
| Boron (B) | 75–150 ppm |
| Iron (Fe) | 75–250 ppm |
| Manganese (Mn) | 5–20 ppm |
| Copper (Cu) | 1–5 ppm |

| | |
|---|---|
| Zinc (Zn) | 25–50 ppm |
| **Carbohydrates** | |
| Fucoidan | 11.6% |
| Alginic acid | 28% |
| Mannitol | 7.5% |
| Laminarin | 4.5% |
| **Amino acids (total 4.4%)** | |
| Alanine | 0.32% |
| Arginine | 0.04% |
| Aspartic acid | 0.62% |
| Cystine | 0.01% |
| Glutamic acid | 0.93% |
| Glycine | 0.29% |
| Histidine | 0.08% |
| Isoleucine | 0.26% |
| Leucine | 0.41% |
| Lysine | 0.16% |
| Methionine | 0.11% |
| Phenylalanine. | 0.25% |
| Proline | 0.28% |
| Serine | 0.08% |
| Threonine | 0.04% |

| | |
|---|---|
| Tyrosine | 0.17% |
| Valine | 0.28% |
| Tryptophan | 0.07% |

Several studies have revealed that the application of ANE not only improves plant growth and yield, but also improves tolerance to abiotic stresses such as nutrient deficiency, salt, drought, thermal and cold stress (Shukla et al., 2019; Shukla et al. 2018; Kumar and Sahoo 2011). Shukla et al*.,* (2018) showed that ANE improves drought tolerance of *Glycine max* by inducing the expression of a stress responsive gene involved in stomatal conductance. The evidence published in this and other reports show that the use of ANE in agriculture presents a promising approach to improve plant growth and impart stress tolerance.

### 2.7.2 *Ascophyllum nodosum* extract and plant diseases:

ANE is a potential elicitor and possesses disease suppressive activities (Khan et al. 2009). The root drench and foliar application of ANE improves growth rates, reduces incidence of pest and diseases, enhances crop yield and improves overall crop quality (Khan et al. 2009). Several published reports (Table 4) suggested that the extract from brown seaweed ANE imparts resistance in plants against various fungal and bacterial diseases, and it also helps plants to overcome from several diseases (Sharma et al. 2014).

Jayaraman et al. (2008) showed that carrot plants sprayed with *A. nodosum* extract significantly reduced the severity of *Alternaria radicina* and *Botrytis cinereal*

by enhanced activity of defensive enzymes (PAL, PPO, PO, chitinase and β-1,3 glucanase). The ANE-treated carrot plants showed higher transcript levels of defense responsive genes such as pathogenesis related protein 1, chitinase and chalcone synthase as compared to the control (Jayaraj et al. 2008). Similarly, Ali et al. (2016) showed that foliar spray of ANE along with alternate application of fungicides significantly reduced the disease incidence of tomatoes caused by *Alternaria solani* and *Xanthomonas compestris* grown in controlled and field conditions (Ali et al. 2016). Application of ANE showed a reduction in disease incidence in field grown tomatoes as well as enhanced yield up to 42 % as compared to the control. ANE application showed higher activity of different defense enzymes such as chitinase, glucanase, phenylalanine ammonia lyase activity, polyphenol oxidase, peroxidase. Tomato plants sprayed with ANE accumulated higher phenolic content as compared with control (Ali et al. 2016).

The application of a combination of ANE with fungicide (chlorothalonil) showed reduction of *Alternaria cucumerinum, Didymella applanata, Fusarium oxysporum and Botrytis cinereal* on greenhouse grown cucumber plants (Jayaraman et al. 2011). Combined spray and drench with commercial extract from *A. nodosum* showed significant reduction in disease incidence of four fungal pathogens. In this study, the fungicide application alternated with commercial extract was highly effective and found to be the best treatments. The cucumber plants treated with commercial extract have shown the increased level of various defense related enzymes (chitinase, β-1,3 glucanase, peroxidase, polyphenol

oxidase, phenylalanine ammonia lyase and lypooxigenase) (Jayaraman et al. 2011).

Similarly, Subramanian et al. (2011) showed that the root irrigation of *Arabidopsis thaliana* with different organic fractions of *A. nodosum* extracts reduces the progression of *Pseudomonas syringae pv tomato* and *Sclerotinia sclerotiorum* infection by inducing jasmonic acid dependent systemic resistance (Subramanian et al. 2011). The root irrigation of onions with seaweed extract (*A. nodosum*) showed reduction of downy mildew caused by *Peronospora destructor*. The seaweed extract application significantly reduces the severity of downy mildew in onions and showed higher growth and yield of onion (McGeary and Birkenhead 1984; Dogra and Mandradia 2012).

According to Wite et al. (2015), the application of commercial extracts from *Durvillaea potatorum* and *A. nodosum* significantly reduces the progression of disease caused by *Plasmodiophora brassicae* due to the presence of laminarins (polysaccharide) and exogenous growth regulators *in broccoli.* At the initial stage of infection, seaweed application reduced the number of plasmodia by 55 % in the root hairs and at the later stage, plasmodia were reduced by 84 % in the root cortical cells (Wite et al. 2015).

The disease suppression effect of ANE have been studied on a number of pathogens. However, its effect on powdery mildew has not been studied. Therefore, the objective of this study was to test the effect of ANE on powdery mildew of strawberry in the greenhouse and under field condition.

**Table 4. *Ascophyllum nodosum* extract induce plant's resistance against pathogens**

| Product | Crop | Pathogen name | Disease | Function | Reference |
|---|---|---|---|---|---|
| *Ascophyllum nodosum* extract (Acadian Seaplant) | Carrot | Alternaria radicina and Botrytis cinereal | Black rot, Botrytis blight | Induces expression of defense related genes or proteins | Jayaraj et al. 2008 |
| *A. nodosum* extract (Acadian Seaplants) | Tomato | *Alternaria solani*, *Xanthomonas campestris pv vesicatoria* | Alternaria blight, Bacterial leaf spot | Reduces incidence of diseases in plants by the upregulation of JA/ethylene pathway | Ali et al. 2016 |
| *Stimplex*® (Acadian Seaplants) | *Cucumber* | *Alternaria cucumerinum, Didymella applanata, Fusarium oxysporum,* | Alternaria blight, Gummy stem blight, Fusarium root and stem rot, Botrytis blight | Stimplex reduces the disease by activating different-related enzymes and accumulation of second dary metabolites | Jayaraman et al. 2010 |

| | | | | | |
|---|---|---|---|---|---|
| | | *Botrytis cinereal* | | | |
| *A. nodosum extract* (Acadian Seaplants) | Arabidopsis | *Pseudomonas syringae, Sclerotinia sclerotiorum* | Bacterial speck, Stem rot | Reduces the development of diseases by regulating jasmonic acid dependent gene expression | Subramanian et al. 2011 |
| *A. nodosum extract* (Acadian Seaplants) | Onion | *Peronospora destructor* | *Downy mildew* | Reduces the disease severity of leaves and bulbs of onions and enhances the growth and yield of onions. | Dogra et al. 2012 |
| Seasol Commercial® | Broccoli | *Plasmodiophora brassicae* | Clubroot | Stimulation of resistance mechanism related to laminarin and effect of exogenous growth regulators | Wite et al. 2014 |

# 3.0 MATERIALS AND METHOD

## 3.1 Plant material:

A susceptible cultivar of strawberry “Honeoye” was purchased from G.W. Allen Nursery (Centreville, NS, Canada). Seedlings were initially kept in a cold room for two days and later transferred to room temperature to gradually maintain the transition from frost to natural temperature. Seedlings were grown in 3 Litre pots containing PRO-MIX® (Premier Tech, QC, Canada) in a greenhouse with 16/8-hour day/night photoperiod at temperature 22 ± 2 ˚C. Fertilizer N-P-K (20-20-20) was applied every three weeks with 100 ml/plant. Plants were grown in the greenhouse for one month for further experiments.

## 3.2 Maintenance of pathogen:

Strawberry plants were purchased from the Annapolis Valley and the natural infection of powdery mildew was maintained at Greenhouse of Dalhousie University, Faculty of Agriculture, Truro, NS. The fungus *Podosphaera aphanis* (*Syn Sphaerotheca macularis*) was maintained in a growth chamber (16/8-hour photoperiod (day/night) with 22 ± 2 ºC). The pathogen was confirmed based on the spore morphology.

## 3.3 Preparation of ANE and treatment:

*Ascophyllum nodosum* extract (ANE) powder was obtained from Acadian Seaplants Limited (Dartmouth, Nova Scotia, Canada), and was used in all experiments. A stock solution of five percent (5 gm of ANE powder dissolved in 100 ml of Milli Q water) was prepared and stored in 4 ˚C until further use. Three

concentrations of ANE (0.1 %, 0.2 % and 0.3 %) were prepared by using the stock solution supplemented with 0.02 % Tween®20. A control treatment with Milli Q water containing 0.02 % Tween®20 was also prepared (described in Table 5).

**Table 5. Different concentrations of ANE evaluated for their efficiency against Powdery mildew of Strawberry**

| **Treatment** | **Concentration of *Ascophyllum nodosum extract* (ANE) (%)** | **Tween® 20 (%)** | **Other** |
|---|---|---|---|
| C | - | 0.02 | Milli Q water (Control) |
| T1 | 0.1 | 0.02 | |
| T2 | 0.2 | 0.02 | |
| T3 | 0.3 | 0.02 | |

## 3.4 ANE treatment and plant inoculation:

The strawberry plant was sprayed with different concentration of ANE twice: the first treatment on the first day of the experiment, followed by a subsequent treatment on the fifth day. Control plants were sprayed with MilliQ water containing 0.02% Tween®20. The strawberry leaves were fully covered on both abaxial and adaxial sides with the treatment solutions. The plants were sprayed until dripping and treatment was applied by a hand sprayer.

Different methods were used for fungal inoculation in this study including conidial suspension, rubbing and tapping (dusting). The conidial suspension had some inconsistent results; some researchers have observed successful inoculation with this method; however, a couple of studies have shown that inoculation with conidial suspension resulted in spore clumping, poor spore deposition and consequently, poor fungal growth (Nicot et. al., 2002). In this study, the tapping method was found to be the best and most reliable method and was further used for all experiments.

The strawberry plants infected with powdery mildew were used as inoculum for further inoculation. Forty-eight hours after the second ANE treatment, the strawberry leaves were inoculated by tapping spores from infected leaves (for inoculation the infected leaf was held securely between the thumb and forefinger of one hand and the leaf was gently tapped with the other hand over the healthy leaves) as described by Li et al. (2017) with minor modification. Inoculated plants were put in a plastic bag to maintain high humidity and shifted to a growth chamber (16/8-hour photoperiod (day/night) with 22 ± 2 ºC) for further experiments (Figure 5). Each treatment consisted of six plants with three biological replications.

**Figure 5. *P. aphanis* inoculated one-month old strawberry plants in transparent bags to maintain high humidity.**

## 3.5 Effect of ANE on spore germination:

The detached leaf assay was performed as described by Feechan et al. (2015) with minor modifications. The youngest, healthy, fully expanded leaf of each strawberry plant sprayed with different concentration (0.1 %, 0.2 % and 0.3 % supplemented with 0.02 % Tween® 20) was detached from the plant after 48 hours of second treatment and inoculated with spores of *P. aphanis.* A moist chamber was prepared with 2-3 filter paper and autoclaved to avoid any cross contamination. After inoculation, the leaves were placed in the moist chamber and incubated for 48 hours at 25 ºC to maintain high humidity (Figure 6). Forty-eight hours post-inoculation, the leaves were dechlorophyllized with a solution of one-part acetic acid to three parts ethanol (1:3) overnight or until the leaf was completely decolorized and rinsed in several changes of sterile distilled water. The clear leaves were stained with 10 mL of trypan blue solution containing equal parts of lactic acid: phenol: distilled water and glycerol (1:1:1:1) and 2 mg of trypan blue for not more than 1 hour. After trypan blue staining, the leaves were rinsed several times with sterile distilled water. For microscopic observation, the leaves were mounted on slides with 50 % glycerol. Microscopic observation was done by using

a light microscope (40 x objectives; Olympus BX51, Japan). Five leaves per treatment were examined, with three replications and number of spores was counted.

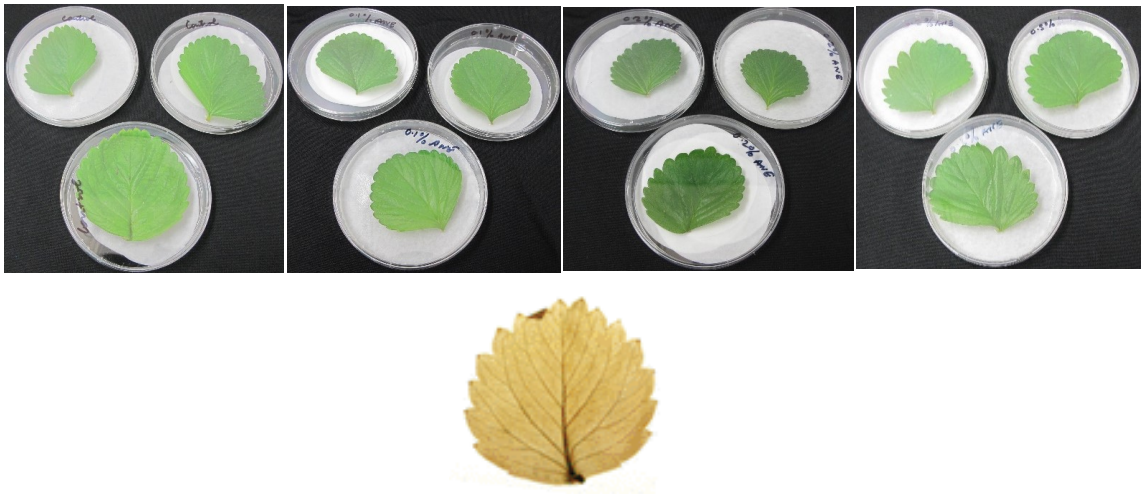

**Figure 6. Detached leaf assay for spore germination.** Strawberry leaves were incubated in moist chamber after inoculation with *P. aphanis* at 25 ºC. The leaves were dechlorophilised after 48 hours of incubation for trypan blue staining.

**3.5.1 Effect of ANE on development of powdery mildew on leaf surface:**

Since powdery mildew is caused by a biotrophic fungal pathogen which cannot grow on artificial nutrient media, the inoculum was maintained on the host plant. For detached leaf assay, 2 % water agar amended with 0.025 % Benzimidazole to prevent early leaf senescence. A water agar assay with detached leaves was done as described by Bieri et al., (2003) with minor modifications. The youngest, healthy, fully expanded leaf of each strawberry plant sprayed with different treatments (0.1 %, 0.2 % and 0.3 % supplemented with 0.02 % Tween® 20) was detached with petiole from the plant and inoculated with spores of *P. aphanis* by the tapping

method. The petioles of the inoculated leaves were inserted in 2 % water agar amended with 25 ml/L of Benzimidazole (to minimize the senescence of leaf). The leaves were incubated at 16/8-hour photoperiod (day/night) with 22 ± 2 ºC for 7 days after inoculation and symptoms were observed, the individual leaves were measured for infected leaf area using ImageJ software (Figure 7). The leaves were suspended in sterile distilled water to compare the number of conidia in control and treatments by haemocytometer.

**Figure 7. Detached leaf for the measurement of leaf area infected using Image J software.** Standard scale was used as a reference for measurements.

## 3.6 Whole plant experiment:

For whole strawberry plant study, the plants were kept and maintained in growth chamber, the experimental setup was completely randomized with six plants in each treatment. Three different concentrations of ANE (0.1 %, 0.2 % and 0.3 % supplemented with 0.02 % Tween®20) and one control (Mili Q containing 0.02 % Tween®20) were sprayed on one-month old strawberry plants at two time points, day 1 and 5. Forty-eight hours after the second treatment, the leaves of the

strawberry plants were inoculated by tapping spores from previously maintained inoculum as described by Li et al. (2017). The plants were closed in plastic bags with moisture to maintain the high relative humidity. The plants were observed daily for visual sign of disease. Disease severity observation was recorded on the 7$^{th}$ and 15$^{th}$ day after inoculation by using a five-point scoring scale (0, 1, 2,3 and 4) (Table 6). Disease severity was calculated by using the following formula described by (Singh and Prithiviraj 1997).

$$DS = \frac{[\{\text{Sum of ratings (0-5 scale}\}]}{(\text{Maximum possible score x Total number of leaves examined})} \times 100$$

**Table 6. Disease severity rating based on infected strawberry leaf area as described by Ali et al. (2016)**

| Infection Rating | Leaf Area Infection (%) |
|---|---|
| 0 | No infection |
| 1 | 1-24 |
| 2 | 25-49 |
| 3 | 50-74 |
| 4 | >75 |

## 3.7 Preparation of plant extract for Total Phenolic and Total Flavonoid content:

For the quantification of secondary metabolites and protein content, leaf samples were harvested at 24, 48, 72, 96 and 120 hours-post-inoculation. The leaf samples were also harvested from the uninoculated ANE sprayed plants at the same time point. The samples were frozen in liquid nitrogen and homogenized with a mortar

and pestle. The leaf samples were brought to the laboratory in an ice-box to maintain low temperature, and samples were homogenized with a cold mortar and pestle in a biosafety hood. The fine frozen powder was immediately suspended in 20 ml of solvent (80 % methanol) and centrifuged at 10,000 rpm for 15 minutes. After centrifugation, the supernatant was transferred into a new 50 ml falcon and the pellet was again added with 10 ml of solvent and centrifuged at the same speed for 10 minutes. The clear supernatant was again transferred into the same tube containing the supernatant for total phenolic content and total flavonoid content calculation. The extract was placed in ice to maintain the cold temperature (Ainsworth and Gillespie 2007).

## 3.8 Preparation of plant extract for PAL, PPO and PO:

To determine the PAL activity in response to powdery mildew the leaf samples were harvested as described above. The leaf samples were homogenized with a cold mortar and pestle, and fine powder was suspended in 10 ml of 25 mM borate buffer (pH 8.8) containing 0.5 g/L polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4° C and the pellet was discarded. The supernatant was centrifuged at 10,000 rpm for 10 minutes at 4° C and the clear supernatant was used as the crude enzyme extract. The activity of phenylalanine ammonia lyase was determined spectrophotometrically by measuring the amount of cinnamic acid formed using L-phenylalanine as the substrate (Subramanian et al. 2011).

For PPO and PO activity, the leaves were harvested at the same time points as above and leaf samples were homogenized with a cold mortar and pestle, and

the resultant fine powder was dissolved in 5 ml of 0.05 M sodium phosphate buffer (pH 6.0) containing 5% polyvinylpyrrolidone (PVP) (w/v). The homogenate was vacuum-filtered and centrifuged at 10,000 rpm for 15 minutes at 4° C, and the pellet was discarded. For standard, 0.1M catechol was used. The clear supernatant was used as the crude enzyme extract. The absorbance was taken for 5 minutes at 30 second intervals and the values were calculated per minute at 546 nm, and for PO the observation was taken at 470 nm and 2 % $H_2O_2$ and guaiacol (Sigma) was used in place of catechol at every 30 second intervals for 5 minutes (Ngadze et al. 2012).

**3.9 Determination of secondary metabolites:**

The total phenolic content was determined by using the Folin Ciocalteu assay protocol described by John et al. (2014) with minor modification. An aliquot (1 mL) of plant extract or standard solution of gallic acid (1, 2, 3, 4, 5, 6, 7, 8 and 9 µg/mL) was added to a 25 mL volumetric flask containing 9 mL of distilled water. A reagent blank was used with distilled water. 1 mL of 0.2 N Folin-Ciocalteu phenol reagent was added to the mixture and shaken. Samples were incubated for 5 minutes at room temperature. After 5 minutes, 10 mL of 7 % sodium carbonate ($Na_2CO_3$) solution was added to the mixture. The mixture was then incubated for 90 minutes at room temperature. After incubation, the absorbance against the reagent blank was determined at 550 nm with a UV-Visible spectrophotometer. Total phenolic content was expressed as mg Gallic acid Equivalents (GAE) (John et al. 2014, Ainsworth and Gillespie 2007) (Figure 8).

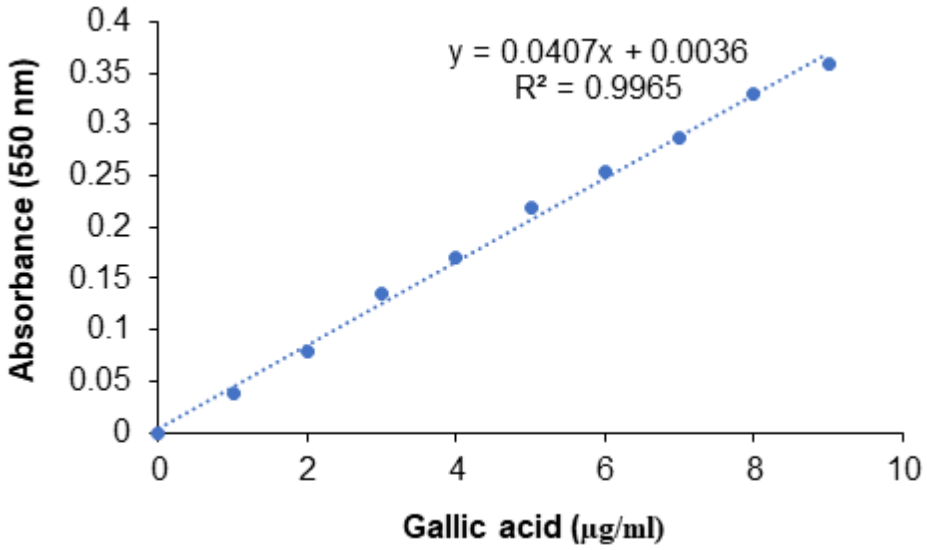


**Figure 8. Standard curve for Total Phenolic Content**

Total flavonoid content was measured using the protocol described by John et al. (2014) by the aluminum chloride colorimetric assay with minor modifications. An aliquot (1 mL) of plant extract and standard solutions of quercetin (0, 10, 20, 30, 40, 50, 60, and 70 µg/mL) was added to a 10 mL volumetric flask containing 4 mL of distilled water. Then, 0.3 mL of 5 % sodium nitrite ($NaNO_2$) was added to the flask and after 5 minutes of incubation at room temperature, 0.3 mL of 10 % aluminium chloride ($AlCl_3$) was added. After five minutes, 2 mL of 1 M sodium hydroxide (NaOH) was added and the volume was adjusted to 10 mL with distilled water. The solution was vortexed, and absorbance was taken against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE) (John et al. 2014) (Figure 9).

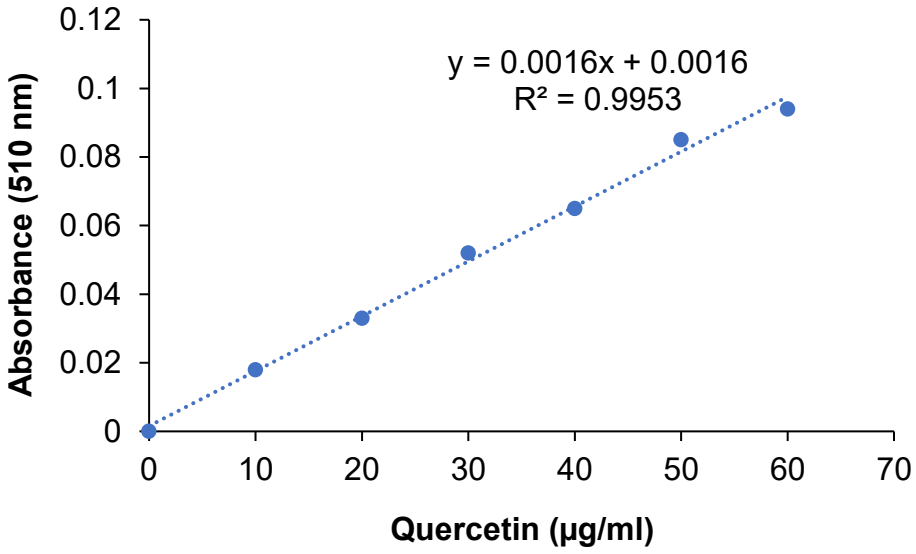


**Figure 9. Standard curve for Total Flavonoid Content**

### 3.10 Activities of defense enzymes in response to disease:

The activities of different defense-related enzymes in ANE-sprayed strawberry plants were evaluated in response to powdery mildew infection with the control.

#### 3.10.1 Phenylalanine ammonia lyase, PO and PPO activity:

PAL enzyme plays an important role in phenyl propanoid pathway, the effect of ANE on PAL activity was observed. For estimation of PAL, leaf samples were harvested at 24, 48, 72, 96 and 120 hours of post-inoculation. The reaction mixture contained 200 µl of enzyme extract and 800 µl of 15mM L-phenylalanine in 25mM borate buffer. The reaction mixture was incubated at 37 °C for 1 hour and the reaction was stopped by adding 50 µl of 5M HCl. The amount of cinnamic acid was calculated using a standard graph of cinnamic acid and expressed as µg $g^{-1}$ FW of leaf (Figure 10).

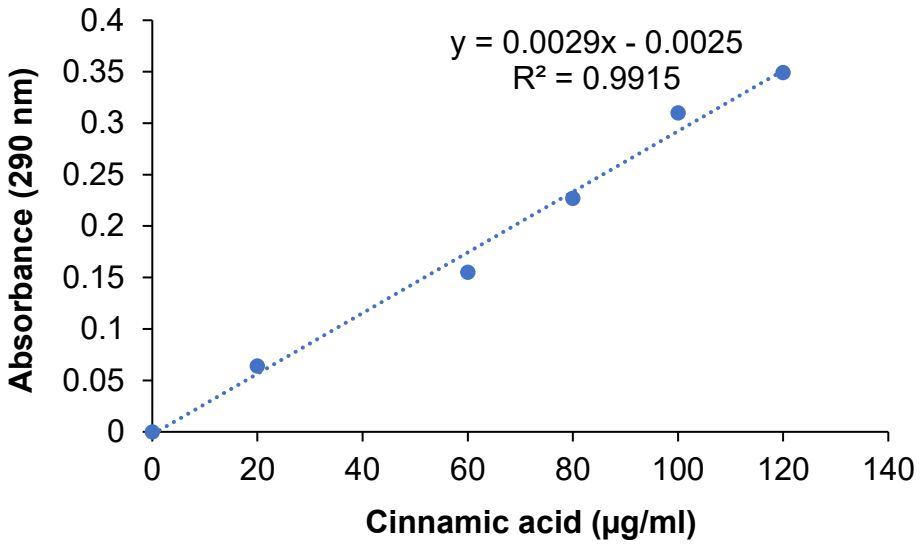


**Figure 10. Standard curve for PAL enzyme**

PO activity was determined as described by Ngadze et al. (2012) with minor modifications. The harvested leaf samples were weighed and homogenized in liquid nitrogen by using a mortar and pestle. The homogenates were mixed with 5 ml of 0.05 M sodium phosphate buffer (pH-6.0) containing 5% polyvinylpolypyrrolidone (PVP) w/v. The homogenate was then filtered through a vacuum filter and centrifuged at 13,000 rpm for 5 min at 4°C. One ml of supernatant was transferred in to a new tube with 2.9 ml of 0.05 M of sodium phosphate and 1 ml of 0.1 M guaiacol. PO activity was assayed using guaiacol as substrate and was observed at 470 nm for 4 min at every 20 sec intervals. The PO activity was expressed as absorbance $s^{-1}$ $g^{-1}$ fresh weight of tissue.

Polyphenol oxidase (PPO) activity was estimated as described by Ngadze et al. (2012). The leaf samples (as harvested for PO activity) were mixed with 5 ml of 0.05 M sodium phosphate buffer (pH-6.0) containing 5% polyvinylpolypyrrolidone (PVP) w/v. The homogenate was then filtered through a

vacuum filter and centrifuged at 13,000 rpm for 5 min at 4°C. One ml of supernatant was transferred in to a new tube with 2.9 ml of 0.05 M of sodium phosphate and 1 ml of 0.1 M catechol. PPO activity was assayed using catechol as substrate and was expressed as catechol equivalents. The absorbance was recorded at 546 nm for 4 min at every 20 sec intervals. The values were presented as absorbance $s^{-1}$ $g^{-1}$ fresh weight of tissue.

## 3.11 FIELD TRIAL:

Field experiments with strawberry plants were carried out at Balamore Farm Ltd., Great Village, NS. The experiments were designed as Randomized Complete Block Design with four replications in each treatment (fifty plants per treatment with six buffer plants). Stock solution of ANE (5%) was prepared and transported to the field with water cans to prepare the working solutions (0.1, 0.2 and 0.3%) for spray. The sprayer was a single nozzle with adjustable tip and was a $CO_2$ pressured with two cylinders. The ANE-treated and control strawberry plants were observed for powdery mildew incidence and severity. For disease assessment, a visual foliar disease scale 0-4 was used (0=no infection, 1=Partial infection on lower surface (lower leaf surface partially covered with pathogen) 2= Complete infection on lower surface (lower leaf completely covered with pathogen), 3= Complete infection on lower and partial infection on upper surface (lower leaf completely covered with pathogen and upper leaf surface partially covered with pathogen) and 4= Complete infection on both lower and the upper leaf surfaces (both leaf surfaces were completely covered with pathogen). Disease was scored every week in the morning after the dew had dried (usually between 10 am-12 am).

During the 2017 field trial, blocks were selected from the existing plots in which the strawberry plants were planted and maintained by the grower. Plots of fifty plants with buffer plots of six plants were marked out in first week of June 2017. Treatments were applied biweekly until the end of the season.

### 3.11.1 Disease Incidence and severity:

The experiment started on the of 7$^{th}$ June 2017 with the initial treatment as a foliar application and treatments continued at fifteen days intervals. The strawberry plants were sprayed with ANE (0.1 %, 0.2 %, and 0.3 %) and water (control) supplemented with 0.02 % Tween 20® until dripping. The strawberry plants were continuously observed for the natural occurrence of powdery mildew. After the first incidence of the disease, disease incidence was recorded. For disease severity data, observations were recorded every seven days usually on Tuesdays (based on weather forecast) for 4 weeks. After that, the disease was minimized due to colder temperature. For treatment application, the single nozzle sprayer with adjustable spray tip was used.

## 3.12 Statistical analysis:

Lab experiments were set up in a completely randomized design with three replications. Field trials were set up by using RCBD (Randomized Complete Block Design). The data was analyzed using “Proc. mixed procedure”, with a P-value of ≤ 0.05 of the SAS Institute, Inc. Software version 9.4 (SAS Institute, Inc., Cary, NC, USA). When significant effects of treatments were found, a multiple means comparison was carried out using Tukey’s test, with a 95% confidence interval and $\alpha = 0.05$ to differentiate treatment means.

# 4.0 RESULTS

## 4.1 Effect of ANE on spore germination:

The effect of ANE on *P. aphanis* (syn. *S. macularis*) spore germination was evaluated on detached strawberry leaves sprayed as described in section 3.4. The inoculated leaves were kept in a moist chamber and incubated for 48 hours at 25°C in the dark to maintain high humidity. After 48 hpi (hours post-inoculation), the leaves were dechlorophilized and stained with trypan blue. The spore germination assay was performed in triplicate with fifteen leaves per treatment under a light microscope. For each leaf, five hundred (500) spores were counted and calculated for percent germination. In spore germination assay, ANE sprayed leaves showed a pattern of spore germination in the treatments (Figure 11). Foliar application with 0.2 % ANE significantly reduced the rate of spore germination by 75 %, while in other treatments, the reduction rate in spore germination was observed by 66.3 % in 0.1 % ANE and 54 % in 0.3 % ANE sprayed leaves, as compared with the control.

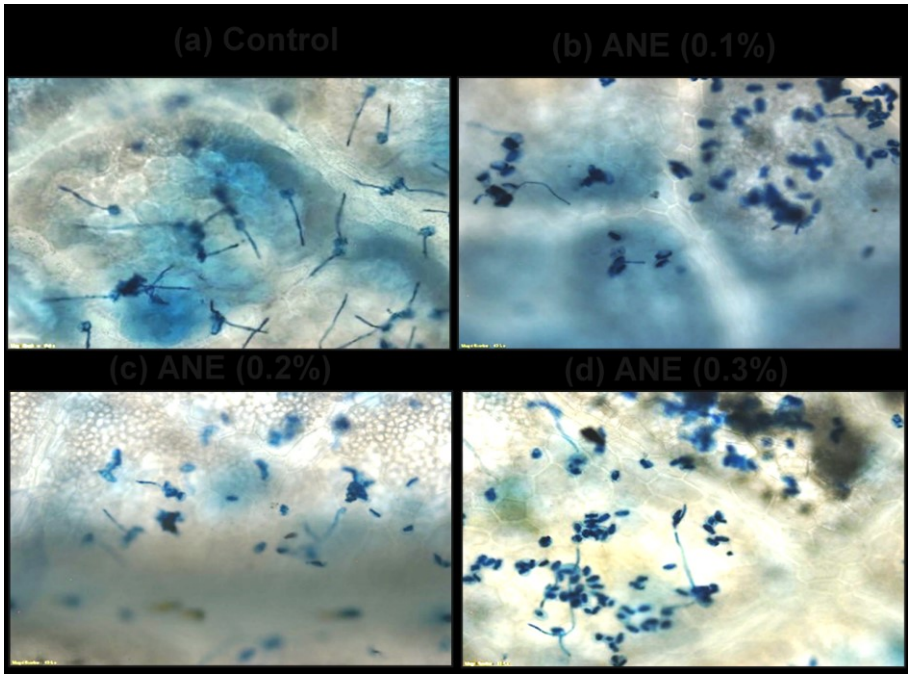


**Figure 11. The effect of foliar spray with ANE on spore germination of *P. aphanis* in detached strawberry leaves**. Microscopic view of germinated spores in Trypan blue stained strawberry leaves sprayed with **(a)** Control**, (b)** 0.1% ANE**, (c)** 0.2% ANE**,** and **(d)** 0.3% ANE.

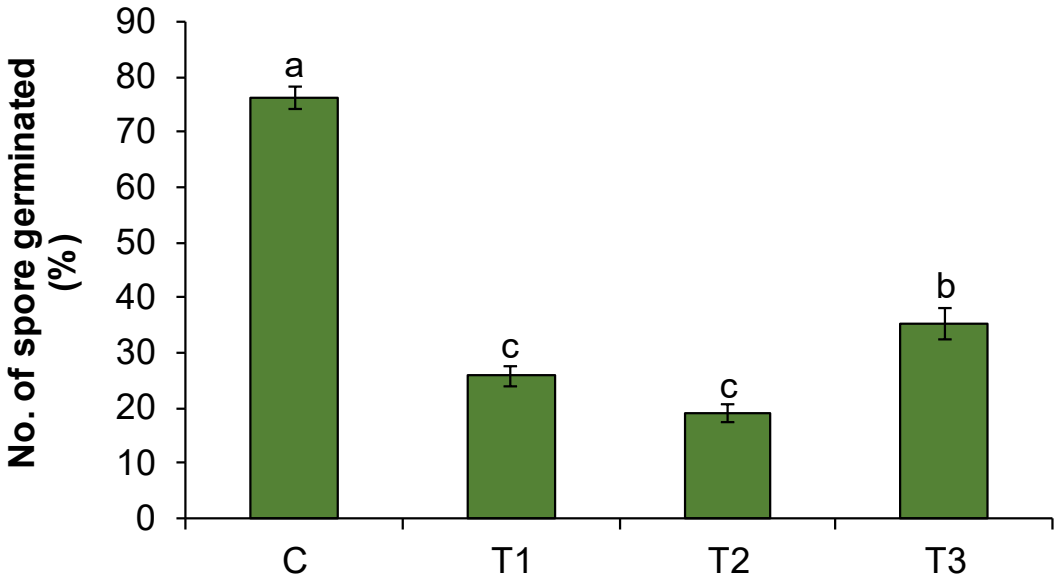


**Figure 11.1. The effect of foliar spray with ANE on percent spore germination of *P. aphanis* in detached leaves.** The graph represents percentage germination of spores of *P. aphanis* in Trypan blue stained strawberry leaves sprayed with **(C)** Control**, (T1)** 0.1% ANE**, (T2)** 0.2% ANE and **(T3)** 0.3% ANE, after 48 hpi. Values were represented as mean± SE (n=5) and marked with different letters indicate significant difference at P≤0.05 probability.

## 4.2 Effect of ANE on powdery mildew development in strawberry leaves grown in growth chamber:

The progression of powdery mildew infection was observed on ANE treated and control strawberry leaves of plants grown in a growth chamber with controlled growth conditions (16/8-hour photoperiod (day/night) with 22 ± 2 ºC). The disease progression was recorded 7 days post-inoculation (dpi).

Leaves sprayed with different concentrations of ANE were observed for the progression of disease. Data on disease development was recorded 7 dpi by

measuring infected leaf area and counting the number of spores by using a haemocytometer. Results found in this experiment were in correlation with the spore germination assay. The results showed that the control leaves had a larger area of infection, while ANE sprayed leaves had less infected area and showed a significant reduction in the progression of powdery mildew by reducing the germination of spores of *P. aphanis* per infected leaves (Figure 12). The leaf area covered with powdery mildew was reduced by 34.36 %, 29.1 % and 42 % in 0.1 %, 0.2 % and 0.3 % ANE treated leaves, respectively, as compared to the control (Figure 12.1, 12.2). The spray of 0.3 % ANE was most effective in reducing the powdery mildew infection in strawberry leaves.

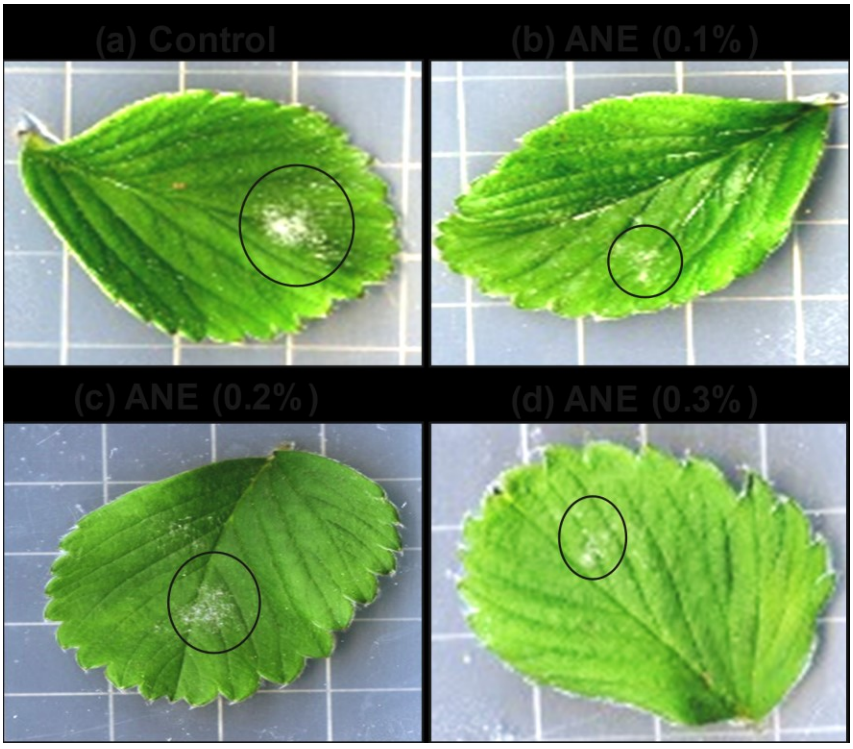


**Figure 12. ANE reduces powdery mildew infection on strawberry leaves.** The foliar spray of ANE reduces powdery mildew on detached leaves of strawberry placed on 2 % water agar sprayed with **(a)** control**, (b)** 0.1% ANE**, (c)** 0.2% ANE and **(d)** 0.3% ANE.

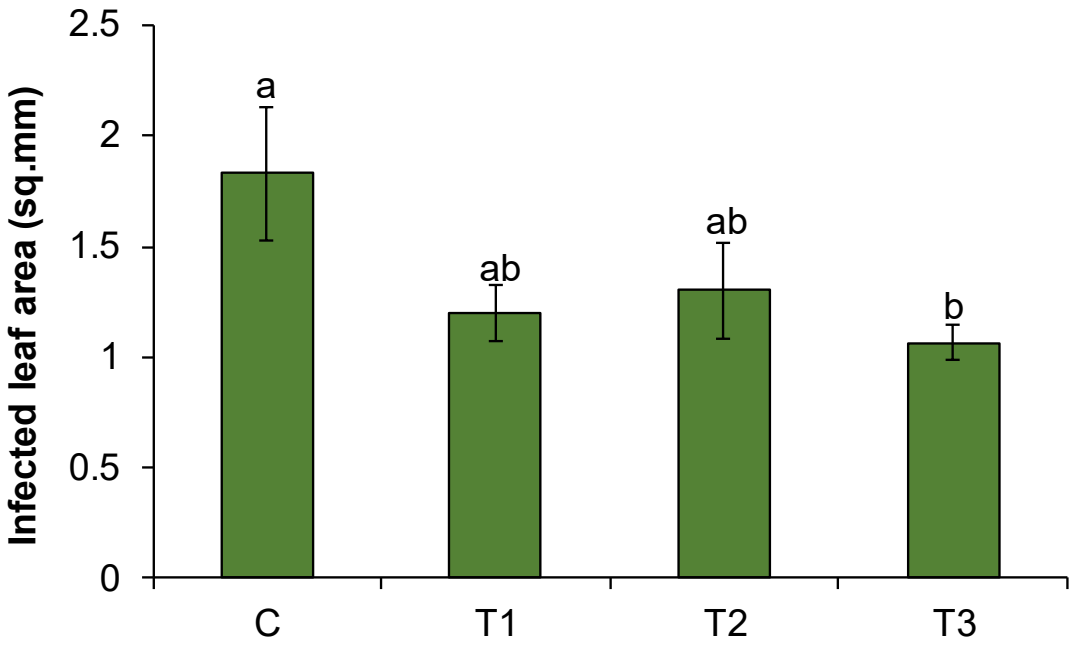


**Figure 12.1. ANE reduces progression of powdery mildew.** The graph represents mean infected leaf area of the detached strawberry leaves sprayed with **(C)** Control**, (T1)** 0.1% ANE**, (T2)** 0.2% ANE and **(T3)** 0.3% ANE, after 7 dpi. Values were represented as mean± SE (n=5) and marked with different letters indicate significant difference at P≤0.05 probability.

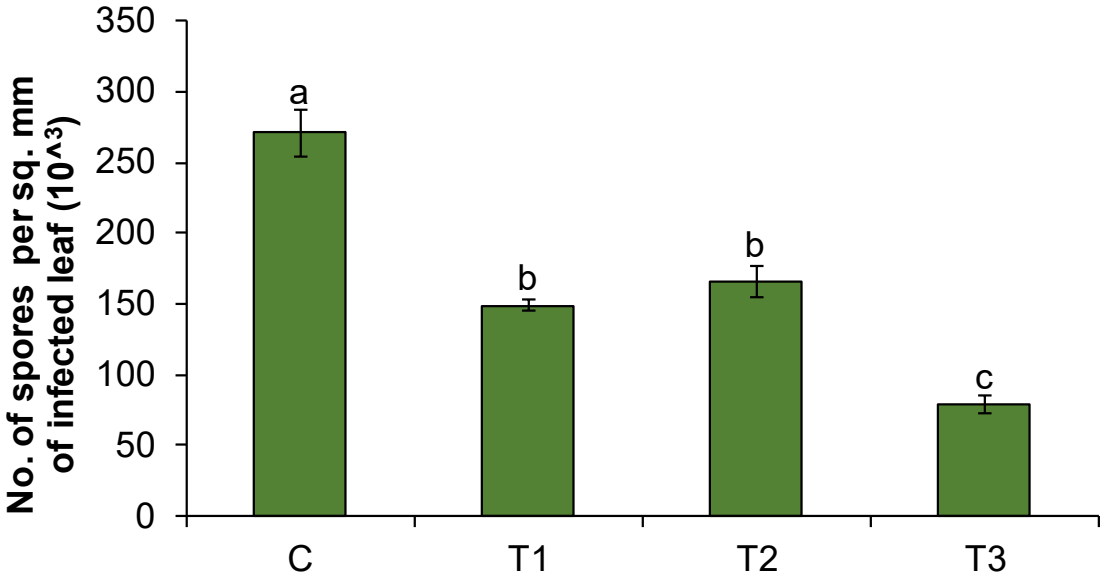


**Figure 12.2. Number of spores per square mm of infected leaf of strawberry** sprayed with **(C)** Control**, (T1)** 0.1% ANE**, (T2)** 0.2% ANE and **(T3)** 0.3% ANE. Values were represented as mean± SE (n=5) and marked with different letters indicate significant difference at P≤0.05 probability.

## 4.3 ANE reduces severity of powdery mildew in growth chamber grown strawberry plants:

The effect of ANE on the severity of powdery mildew in strawberry plants grown under controlled conditions in a growth chamber (16/8-hour photoperiod (day/night) with 22 ± 2 ºC) was evaluated. For disease severity observations, the plants were sprayed twice with different concentrations of ANE, while control plants were sprayed with water. The experiment was repeated in triplicate with six plants in each treatment, and pots were randomly positioned. After 48 hours of the second treatment, plants were inoculated with fungal spores and covered in a transparent plastic bag to maintain a high level of humidity. The plants were observed every

day for visual presence of powdery mildew sign and symptoms on the leaves. The disease severity data was recorded 7 and 15 dpi by scoring the plants based on 0-4 scaling (where 0=no infection, 1= partial infection on lower surface, 2= complete infection on lower surface, 3= complete infection on lower and partial on upper surface, and 4= complete infection on upper and lower surface) depending on the progression of the disease and number of leaves infected. ANE-treated leaves showed reduced disease severity when compared with the control (Figure 13). Seven days post-inoculation, the plants treated with 0.2 % ANE showed maximum reduction in disease progression by 65.7 %, as compared to the control. An increased trend was observed in disease on $15^{th}$ day post-inoculation, the reduction in disease progression in the plants treated with 0.2 % ANE was observed by 71 %, as compared to the control (Figure 13.1). Comparatively, the control plants showed 48 % of increased powdery mildew from Day 7 to Day 15, while the foliar application on strawberry plants with ANE treatments 0.1 %, 0.2 % and 0.3 % ANE, showed an increase of powdery mildew with 32 %, 27 % and 32.4 % infection from Day 7 to Day 15 respectively. Thus, we found that the spray of 0.2 % ANE was the most effective concentration in reducing the progression of powdery mildew. This concentration not only reduced the incidence of powdery mildew, but also prevented its progression on strawberries grown in controlled conditions (Figure 13).

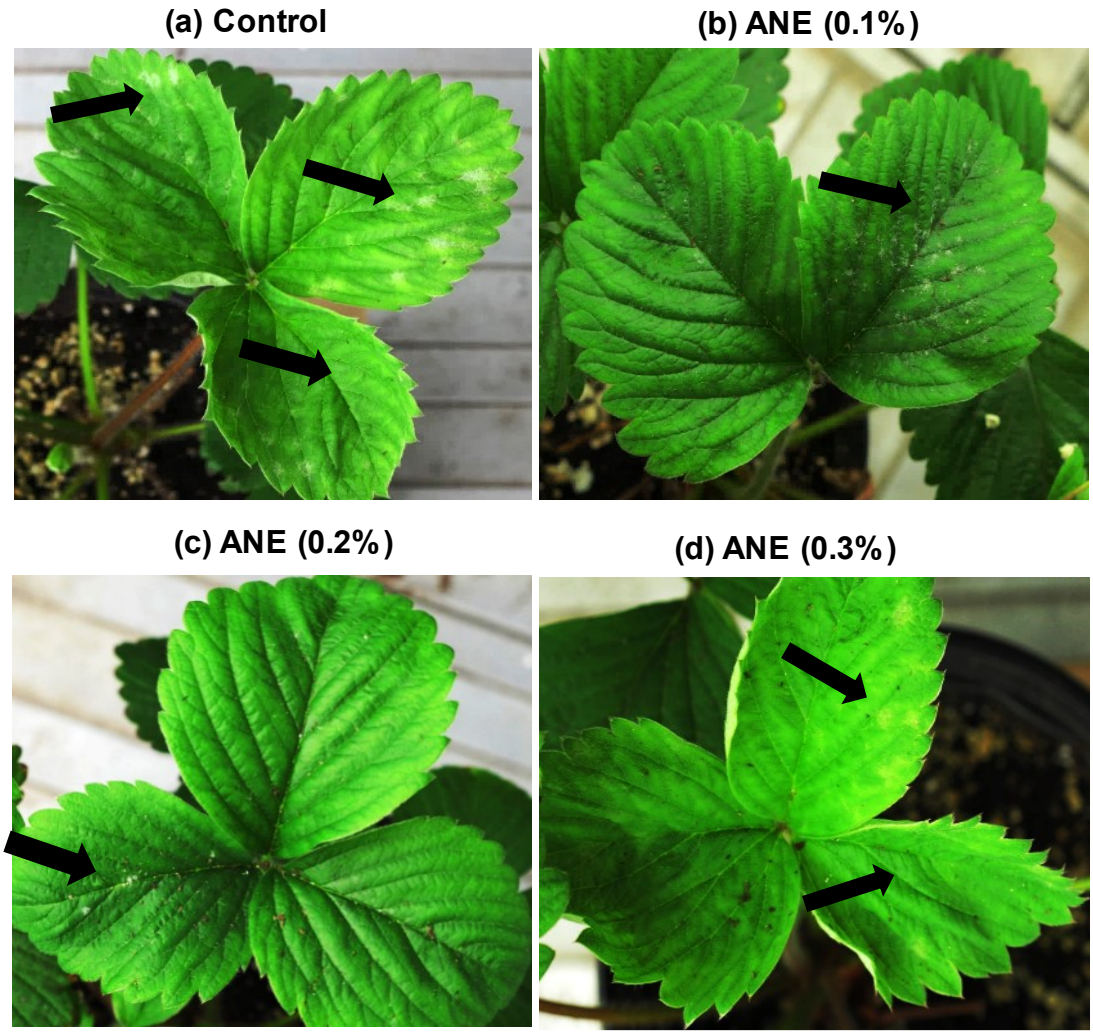


**Figure 13. The effect of foliar spray of ANE on powdery mildew of strawberry grown in green house conditions.** Powdery mildew spores developed on whole strawberry plants sprayed with **(a)** control**, (b)** 0.1% ANE**, (c)** 0.2% ANE and **(d)** 0.3% ANE.

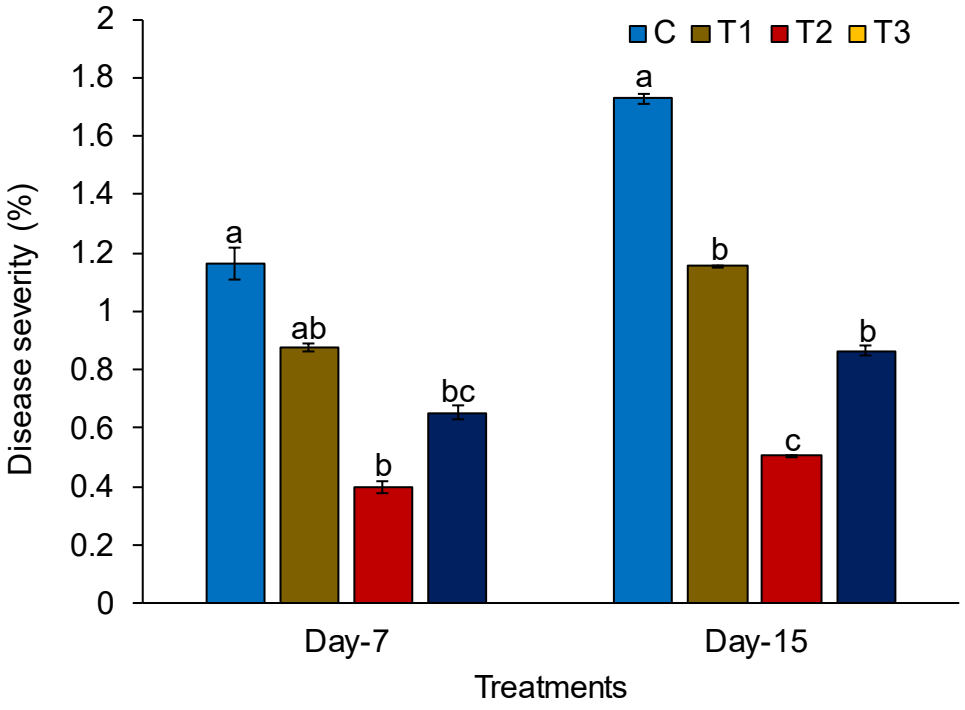


**Figure 13.1. Disease severity on 7th and 15th day post-inoculation with spores of *P. aphanis* in strawberry plants** sprayed with **(C)** Control**, (T1)** 0.1% ANE**, (T2)** 0.2% ANE and **(T3)** 0.3% ANE. Values were represented as mean± SE (n=6 with three biological replications) and marked with different letters indicate significant difference at P≤0.05 probability.

## 4.4 BIOCHEMICAL ANALYSIS OF SECONDARY METABOLITES:

### 4.4.1 ANE induces change in total phenolic content of strawberry leaves infected with powdery mildew:

Plants synthesize a diverse group of phenolic compounds that play an important role in plant defense against pathogens. To understand the mechanism of how ANE reduces powdery mildew infection, we have determined the level of phenolic

content in plants inoculated with fungal spores. For the determination of phenolic levels, the strawberry plants were treated with different concentrations of ANE as described in section 3.7. The results presented in this study showed that plants treated with ANE were synthesizing significantly higher amounts of phenolic compounds as compared with the control. Foliar spray of ANE induces the phenolic content in the leaves of strawberry plants. Inoculation with powdery mildew spores showed further increase in the level of total phenolic content by 30 %, 51.5 % and 14 % in plants sprayed with 0.1 %, 0.2 % and 0.3 % ANE respectively, as compared to the control. The increased biosynthesis of phenolics was observed throughout the time points. The maximum significantly higher phenolic content was observed in plants sprayed with 0.2 % ANE treated after 96 and 120 hours of inoculation (Figure 14).

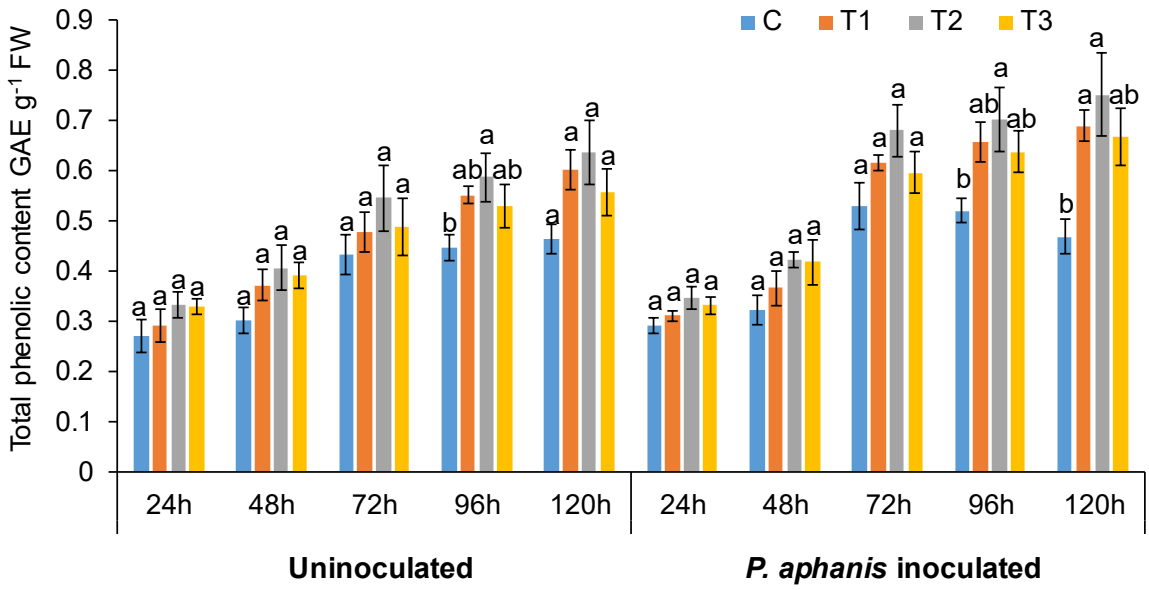


**Figure 14. Effect of ANE on total phenolic content.** This graph represents the content of total phenolics in strawberry leaves sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE), and strawberry leaves sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE) in response to powdery mildew infection. Values represent mean± SE (n=6) and marked with different letters indicate significant difference at P≤0.05 probability.

## 4.4.2 ANE induces change in the flavonoid content in the strawberry leaves infected with powdery mildew:

Flavonoids play an important role in plant defense and are known to have anti-microbial properties. These secondary metabolites are responsible for inducing a hypersensitive response at the infection site, which is an initial defense mechanism of plants against any biotic stress. Spray of ANE showed increase in the flavonoid content in the uninoculated strawberry leaves (Figure 15). The plant primed with ANE spray, further enhanced the flavonoid content in the strawberry leaves upon

further inoculation. Inoculation with pathogen increases the flavonoid content, which was significantly higher after 96 and 120 hours of inoculation in the ANE-sprayed plants, as compared to the control. After 120 hours of foliar spray, 0.2 % ANE showed 104.15 % higher flavonoid content as compared to control, while 0.1 % and 0.3 % ANE showed 24.11 % and 53.87 %, respectively, higher flavonoid content as compared to the control (Figure 15).

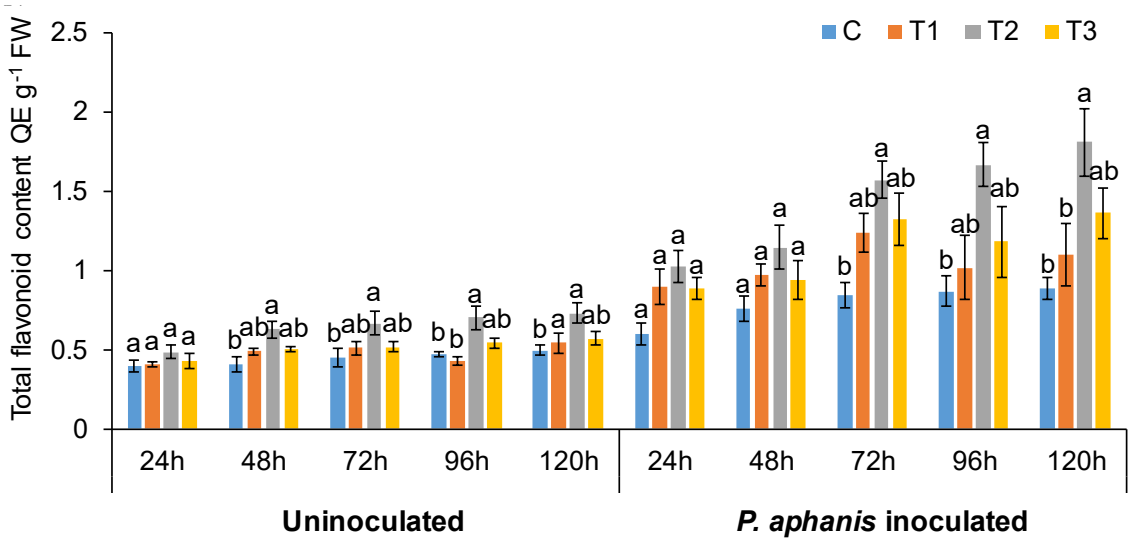


**Figure 15. Effect of ANE on total flavonoid content.** Total flavonoid content of strawberry leaves sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE), and strawberry leaves sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE) in response to powdery mildew infection. The flavonoid content was higher in strawberry leaves treated with ANE and ultimately reduces the severity of powdery mildew. Values represent mean± SE (n=6) and marked with different letters indicate significant difference at P≤0.05 probability.

## 4.5 ANE INDUCE THE BIOSYNTHESIS OF DEFENSE RELATED ENZYMES:

Different defense related enzyme activities were studied in strawberry leaves sprayed with different concentrations of ANE (0.1 %, 0.2 % and 0.3 %) and control plants in response to powdery mildew.

### 4.5.1 ANE induces the activity of phenylalanine ammonia lyase activity (PAL) in ANE sprayed strawberry plants:

Plant protection against pathogens depends on inducible defensive enzyme responses that are generally activated at the time of infection. Phenylalanine ammonia lyase (PAL) is a primary enzyme and plays a crucial role in the phenylpropanoid pathway. PAL participates in the conversion of L-phenylalanine into trans cinnamic acid. To determine the activity of PAL in powdery mildew infected strawberry plants, the plants were treated with ANE (control plants were treated with Milli Q water supplemented with 0.02 % Tween®20). The leaves were harvested after 48 hours of pathogen inoculation as well as from uninoculated leaves at different time points (24, 48, 70, 96 and 120 hours). The elicitors present in ANE induces PAL activity in strawberry leaves. The results showed that pathogen inoculation further increases PAL activity in plants treated with 0.2 % ANE compared with control, after 96 hpi. The PAL activity was significantly induced 110.75 % and 80.66 % respectively, in 0.2 % ANE, after 96 and 120 hpi (Figure 16).

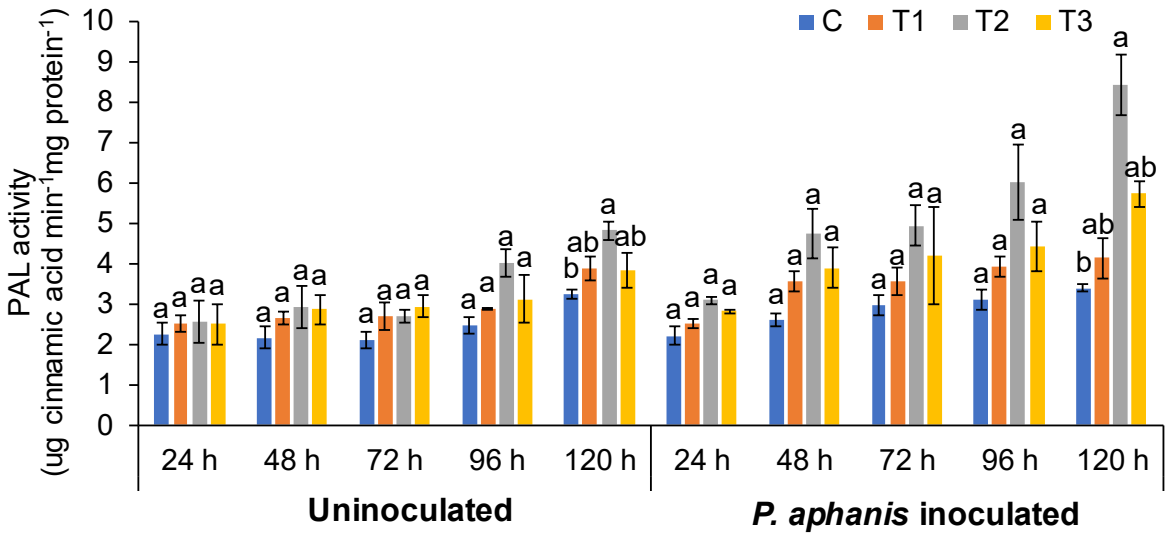


**Figure 16. Effect of ANE on phenylalanine ammonia lyase activity.** This graph represents PAL activity of strawberry leaves sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE), and strawberry leaves sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE) in response to powdery mildew infection. The PAL activity was found higher in strawberry leaves treated with ANE and ultimately reduces the severity of powdery mildew. Values represent mean ± SE (n=6), and different letters indicate significant difference at P≤0.05 probability.

### 4.5.2 ANE induces the activity of polyphenol oxidase activity (PPO) in ANE sprayed strawberry plants:

To determine the activity of phenol peroxidase in strawberry plants sprayed with ANE (and control plants sprayed with Milli Q water with 0.02 % Tween®20), leaves were harvested after 48 hours of inoculation with pathogen as well as from uninoculated leaves at different time points (24, 48, 70, 96 and 120 hours). The

higher induction of PPO activity was observed in 0.2 % of ANE-sprayed strawberry leaves continuously from 24 to 120 hours post-pathogen inoculation (Figure 17).

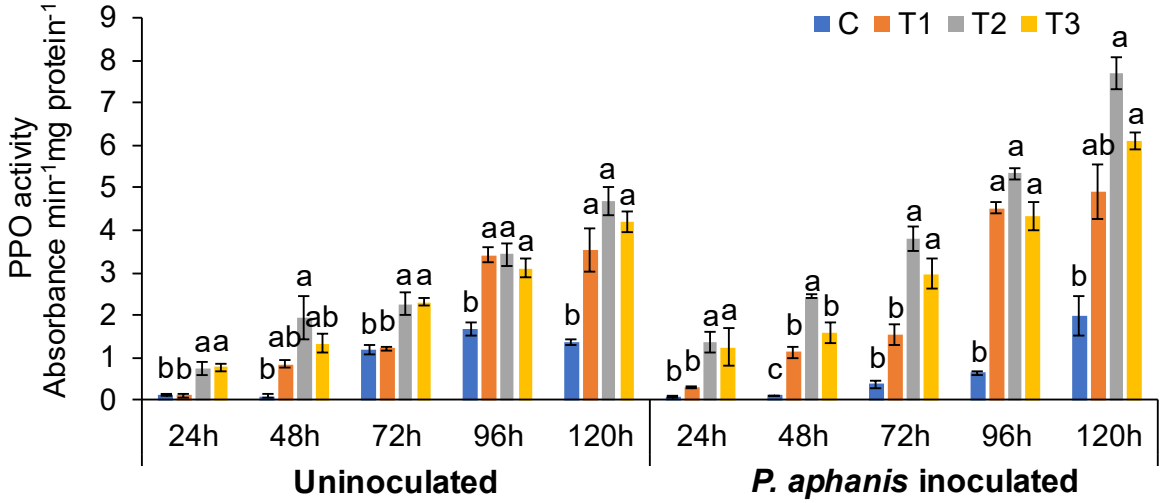


**Figure 17.** The Polyphenol peroxidase (PPO) activities in plants sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE), and strawberry leaves sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE) in response to powdery mildew infection. Maximum PPO activity was observed in strawberry leaves treated with 0.2 % ANE which ultimately inhibited the growth of powdery mildew in strawberry leaves. Values represent mean ± SE (n=6), and different letters indicate significant difference at P≤0.05 probability.

### 4.5.3 ANE induce the activity of peroxidase activity (PO) in ANE sprayed strawberry plants:

Peroxidase activity was also found to be higher in strawberry plants sprayed with ANE in response to the *P. aphanis* infection. The maximum PO activity was observed in the leaves sprayed with 0.2 % ANE (Figure 18).

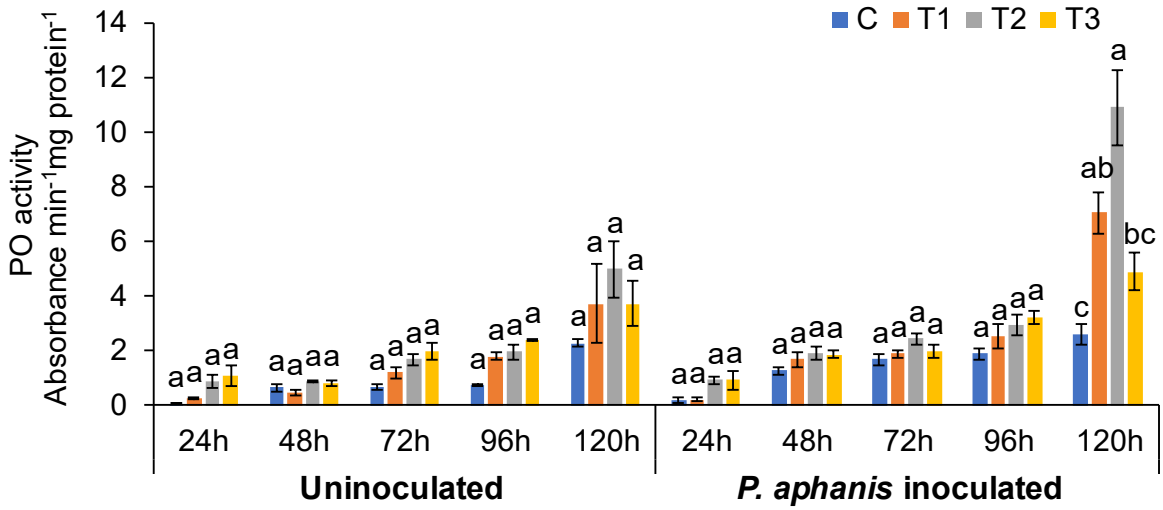


**Figure 18. Effect of ANE on peroxidase activity.** The graph represent peroxidase activity (PO) in strawberry leaves sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE), and strawberry leaves sprayed with **C** (control), **T1** (0.1% ANE), **T2** (0.2% ANE), and **T3** (0.2% ANE) in response to powdery mildew infection. Maximum increased PO activity was found in strawberry leaves treated with 0.2 % ANE at 120 h of inoculation. The increased PO activity was found to reduce the growth of powdery mildew in strawberry. Values represent mean ± SE (n=6), and different letters indicate significant difference at P≤0.05 probability.

## 4.6 FOLIAR SPRAY OF ANE REDUCES DISEASE INCIDENCE AND SEVERITY OF POWDERY MILDEW IN STRAWBERRY PLANTS GROWN UNDER NATURAL CONDITIONS:

The field experiment was carried out at Balamore Farm, Great Village, Nova Scotia, Canada (Figure 19). Three concentrations of ANE (0.1 %, 0.2 % and 0.3 %) were sprayed on strawberry plants until dripping and plants were observed for natural occurrence of powdery mildew. After the occurrence of natural infection of powdery mildew, observations were recorded every seven days after the first occurrence of the disease. The incidence of powdery mildew was 82 % in the control treatment, while the incidence of disease reduced to 68.5 %, 51.5 % and 58 %, in 0.1 %, 0.2 % and 0.3 % ANE treatments, respectively (Figure 20). The 0.2 % ANE treatment showed the maximum reduction of powdery mildew by 37.2 % as compared to control. Based on the scaling of powdery mildew infection of leaves (Figure 21), disease severity was observed in both the treated and control plants (Figure 22). Disease severity of powdery mildew was reduced to 47.2 %, 47.1 % and 51.2 %, respectively, in 0.1 %, 0.2 % and 0.3 % ANE-sprayed plants, as compared to control plants on Day 1 (Figure 22). As time progressed, the disease also progressed in both the control and treated plants from Day 1 to Day 7, but disease progression was significantly reduced by 34.3 % in 0.1 % ANE treated plants and 32 % in 0.2 and 0.3 % ANE treated plants (Figure 23). After Day 14 of disease incidence, reduction in disease was observed in both the control and treated plants.

**Figure 19.** Strawberry field randomly distributed plots with fifty plants in each treatment with four replications.

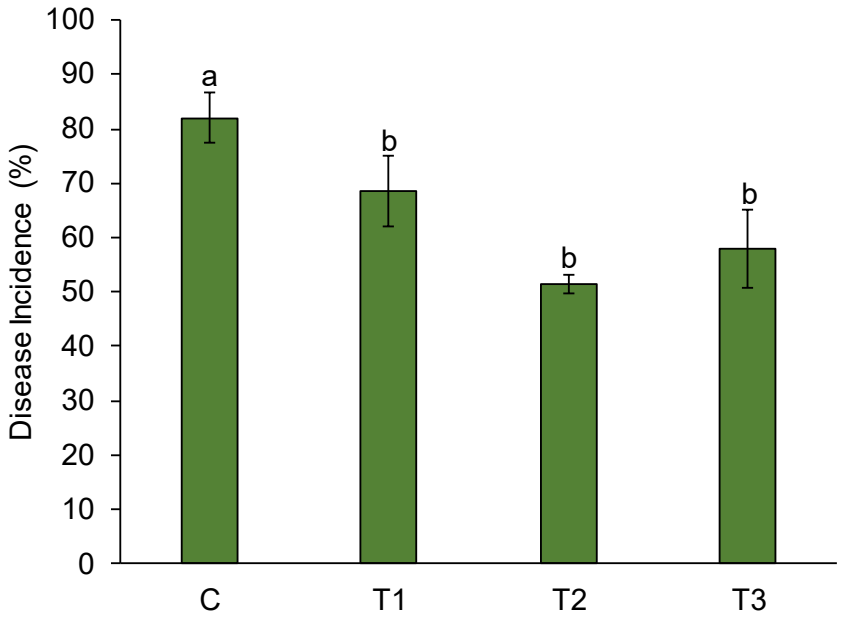


**Figure 20. Natural incidence of powdery mildew on strawberry plants grown in commercial farm, NS, Canada**. Disease incidence (%) of strawberry leaves sprayed with **(C)** Control**, (T1)** 0.1% ANE**, (T2)** 0.2% ANE and **(T3)** 0.3% ANE under field conditions. Maximum reduction was observed in strawberry leaves treated with 0.2 % ANE. Values were presented as mean ± SE (n=50), and different letters indicate significant difference at P≤0.05 probability.


**Figure 21.** Scoring scale for powdery mildew disease assessment on field grown strawberry. The scale is as follows: (1) Partial infection on lower surface, (2) Complete infection on lower surface (3) Complete infection on lower and partial infection on upper surface and (4) Complete infection on lower and upper surface.

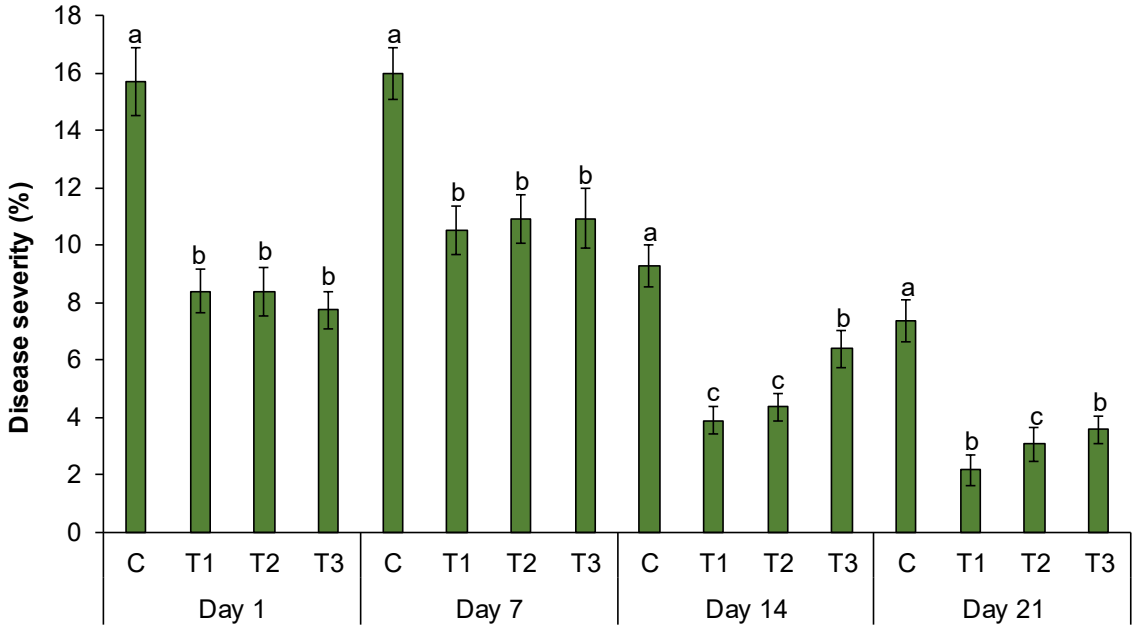


**Figure 22. Disease severity of powdery mildew observed every seven days for one month after the natural occurrence of disease on strawberry plants** sprayed with **C** (control), **T1 (**0.1% ANE)**, T2** 0.2% ANE**,** and **(iv) T3** 0.2% ANE under field conditions. Values were presented as mean ± SE (n=20), and the different letters indicate significant difference at P≤0.05 probability.

# 5.0 DISCUSSION

Strawberry is an economically important crop, and its productivity is reduced by plant diseases. The use of synthetic chemical pesticides for the management of plant diseases have adverse impacts on human health and the environment. Several sustainable alternatives for plant disease management have been proposed. The elicitor molecules present in seaweed extracts enhance plant defenses against various pathogens (Khan et al. 2009; Burketova et al. 2015). Several reports show that *Ascophyllum nodosum* extract acts as a potential elicitor and possesses disease suppressive activities (Khan et al. 2009; Jayaraman et al. 2011). The extracts from *A. nodosum* (ANE) have been known to suppress diseases in several plants such as Arabidopsis, carrot, cucumber and tomato (Jayaraman et al. 2011; Subramanian et al. (2011), Jayaraman et al. (2011); Ali et al. (2016); Abkhoo and Sabbagh (2016)). Previously published reports suggest that ANE induces plant defense systems against broad ranges of pathogens (Khan et al. 2009). The main objective of this research was to determine the role of an extract from the brown seaweed *A. nodosum* in plant defense against powdery mildew of strawberry.

In this study, we showed that the foliar spray of ANE elicited defense responses in strawberry against *P. aphanis*. Microscopic observations revealed that germination of fungal spores was significantly reduced in ANE-sprayed plants. Spores of *P. aphanis* directly enter the epidermal cells and extend its hyphae through to different plant cells, ultimately reducing photosynthetic activity also in addition to causing leaf necrosis resulting in defoliation (Karajeh et al. 2012).

Elicitor molecules including laminarin and fucoidans present in ANE act as pathogen associated molecular patterns (PAMPs) due to their structural similarity to the constituents of pathogen cell walls, and these PAMPs are recognized by host transmembrane pattern recognition receptors (PRRs), which serve as an endogenous signal to activate plant immune responses and prime the plants against disease (Eckardt 2008; Zipfel 2009). Therefore, when there is an actual incidence of *P. aphanis* spores occurring on ANE-sprayed leaves, a reduction in the germination of spores on the leaf surface was observed. The effect of ANE in reducing powdery mildew infection was observed both in the greenhouse as well as in natural field conditions, suggesting that ANE is capable of inducing pathogen resistance irrespective of growing conditions.

Seaweed contains phenolic compounds such as phlorotannins (Craigie 2011). Phenolic compounds present in ANE might be the reason behind reduced spore germination of *P. aphanis.* Similarly, Von Ropenack et al. (1998) showed that soluble and cell wall-bound phenolics from the leaf of barley confers resistance to powdery mildew. Prithiviraj et al. 1997 also showed that Bergenin, a natural compound derived from the plant *Flueggea microcarpa* showed reduced spore germination and antifungal activity against *Fusarium udum* on peas. Anacardic acid, a naturally occurring derivative of salicylic acid was also tested for its efficacy in reducing the progression of several plant fungal pathogens such as *Fusarium udum, Fusarium oxisporum, Alternaria alternate, Curvularia lunata, Alternaria carthami, Alternaria brassicae and Colletotrichum capsici.* The application of anacardic acid at the rate of $0.5\times10^3$ µg/mL was found effective in completely inhibiting spore germination of all fungal pathogens except *F. oxysporum* and *F.*

*udum* (Prithiviraj et al. 1997). Bahadur et al (2008) have shown reduced conidial germination on detached as well as on intact leaves of pea by using a methanol extract of cashew nut shells against powdery mildew of pea. Cashew nut contains a naturally occurring alkyl substituted salicylic acid, anacardic acid. These results suggest that naturally occurring bioactive compounds present in *A. nodosum* reduce germination of *P. aphanis* spores.

The application of ANE induced the expression of several pathogenesis related proteins in response to different pathogen infections in cucumber (Jayaraman et al. 2011). In the present study, the foliar spray of ANE on strawberry leaves reduced the powdery mildew infection by increasing total protein content. Similarly, Inbar et al. (2001) demonstrated that applications of benzothiadiazole (BTH) to tomato plants showed higher protein content in response to pathogen infection.

Strawberry resistance to powdery mildew depends on the intricate network of constitutive and inducible defense responses (Amil-Ruiz et al. 2011). Several secondary metabolites play an important role in a plant's defense against pathogens by causing toxicity to the pathogen, or by acting as a precursor to physical defense systems (Bennet and Wallsgrove 1994). These secondary metabolites form biological chemical barriers and act locally at the early stage of infection (Kliebenstein 2004). The various elicitors induce the accumulation of different secondary metabolites in response to pathogen attack (Zhao et al. 2005). In this study, the effect of ANE on the accumulation of different secondary metabolites in strawberry plants were evaluated in response to powdery mildew infection. Foliar application of ANE increased the phenolic concentration in

strawberry plants in response to powdery mildew infection. Similarly, Stimplex™, a commercial product form of *A. nodosum* showed higher phenolic content in cucumber in response to a different fungal pathogen (Jayaraj et al. 2011). A similar effect of ANE on phenolic content was observed in carrot in response to *Alternaria radicina* and *Botrytis cinereal* infection (Jayaraj et al. 2008). Strawberry plants are a natural source of various phenolic compounds such as ellagitannins and ellagic acid (Aaby et al. 2005; 2007). Foliar application of benzothiadiazole (BTH) and glycine betaine was shown to stimulate production of phenolics such as ellagitannin, ellagic acid, gallic acid derivatives, quercetin and kaempferol conjugates in response to fungal infestations (Gorlach et al. 1996, Karjalainen et al. 2002; Hukkanen et al. 2007). Flavonoids are actively synthesized in strawberry plants, inducing disease resistance (Treutter 2006; Amil-Ruiz et al. 2011). In the present study, the foliar spray of ANE lead to the accumulation of flavonoids in the strawberry plants and suppressed the progression of powdery mildew infection. Several published reports have also found a positive correlation between resistance to plant pathogens and the concentration of flavonoids (Treutter 2006; Amil-Ruiz et al. 2011). Strawberry cultivars with higher flavonoid content were found to be more resistant to pathogen infection (Amil-Ruiz et al. 2011). Thus, elicitors present in ANE stimulate the production of phenolic and flavonoid compounds and contribute to the strawberry's active defense against powdery mildew infection.

The enhanced accumulation of phenolics and flavonoids in the strawberry plants was due to increased activity of defense related enzymes such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase

(PO). Phenylalanine ammonia lyase (PAL), an enzyme involved in secondary phenylpropanoid metabolism, plays an important role in a plant's response to pathogens (Kim and Hwang 2014). In the present study, the bioactive component of ANE elicited the activity of PAL in the strawberry leaves and conferred resistance against powdery mildew. Similarly, PO and PPO, which are involved in the biosynthesis of oxidative phenols, contribute to the plant's active defense against pathogenic infestation (Chen et al. 2000). The application of ANE induced resistance against powdery mildew by inducing the activity of PO and PPO involved in the biosynthesis of polyphenols. The induction of defense related enzymes including PAL, PO and PPO were observed 72 hours post-treatment, and further increased up to 120 hours in ANE-treated plants. Several studies showed that the elicitation of resistance against different fungal pathogens by the application of the seaweed extract has been observed due to increased PAL, PO and PPO activities (in apple, cucumber, carrot and tomato) (Ali et al. 2016; Abouraïcha et al. 2015; Jayaraman et al. 2011, 2008; Hernández-Herrera et al. (2014). In these studies, the increased plant's resistance to different pathogens was marked by the enhanced activity and expression of different defense related genes involved in the accumulation of secondary metabolites.

Under field trials, the foliar spray of ANE reduced the incidence as well as severity of disease. This suggests that ANE reduces the powdery mildew in both the laboratory as well as field conditions. Together these results suggest that ANE elicits defense responses in strawberry plants against powdery mildew by regulating the biosynthesis of secondary metabolites and activities of different defense-related enzymes.

# 6.0 CONCLUSION

In conclusion, this study showed that the extract from *Ascophyllum nodosum* reduces the progression of powdery mildew infection in the laboratory as well as field conditions. The use of extract from ANE for the management of powdery mildew minimizes the use of chemical-based fungicides and provides an environmentally safe and sustainable method for the management of fungal diseases in strawberry and other fruit crops. The results presented here are promising but to realize the full potential of ANE in eliciting the defense response and to implement ANE with disease management programs, a more comprehensive understanding of the mode of action of ANE on plant defense pathways is required. The main objective of this research was to determine the role of extract from brown seaweed *A. nodosum* in plant defense against powdery mildew of strawberry.

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