Screening and Application of Effective Agents for Controlling Sweet Potato Soft Rot Disease

ABSTRACT

Sweet potato soft rot is a major fungal disease during sweet potato storage, and the pathogen is *Rhizopus stolonifer*. The disease is often common in sweet potato production, which can cause sweet potato rot, reduce sweet potato quality and cause huge economic losses. At present, the chemical fungicides commonly used in production to prevent and control sweet potato soft rot have a general effect on the prevention and control of soft rot, and are easy to cause pollution to the environment, which does not meet the development needs of green agriculture. Plant essential oils have been confirmed to have broad-spectrum inhibitory activity, combined with their low toxicity and environmentally friendly characteristics, which has attracted widespread attention from scholars. Exploring the combination of new and efficient plant-derived fungicides and chemical pesticides is a new and effective way to prevent and control sweet potato soft rot. In this paper, *R. stolonifer* was used to investigate the inhibitory effects of 16 chemical fungicides and 9 essential oils and their complex paired with *R. stolonifer*. The results are as follows:

1. In order to clarify the control effect of the existing main common fungicides on soft rot in sweet potato, 16 common fungicides were determined by mycelial growth rate method (Tebuconazole, fludioxonil, procymidone, fluazinam, pyrazoxystrobin, prochloraz, kresoxim-methyl, azoxystrobin, prothionazole, octreotide, boscalid, difenoconazole, thifluzamide, carbendazim, pyraclostrobin, and triadimenol). The bacteriostatic activity of Rhizopus creeping was the best inhibitory effect of three fungicides, fluazinam, fludioxonile and tebuconazole, and EC_{50} were 6.418 μg/mL, 2.509 μg/mL and 692 μg/mL, respectively.

2. In order to clarify the prevention and control effect of plant essential oils on sweet potato soft rot, nine essential oils were determined by essential oil fumigation (Oranges, citronella, garlic, stone calamus, sweet orange, mugwort leaf, sandalwood, artemisia annua, and cedar). The inhibitory effect on Rhizopus creeps, among which the inhibition rate of calamus and orange essential oil was as high as 91%.

3. In order to clarify the optimal combination of fungicide and plant essential oil, the indoor control effect of the combination of fludioxonile and calamus essential oil on soft rot in sweet potato was determined by fungus cake inoculation, and the combination of fludioxonile and calamus essential oil could reduce the infection of sweet potato by *Rhizopus* creeps.

In summary, this experiment provides a theoretical basis for the combination of chemical fungicides and plant-derived fungicides in the prevention and control of sweet potato soft rot, and also provides a theoretical basis for the future development of plant essential oils into green and efficient control agents for the prevention and control of sweet potato soft rot during storage.

Key words: Sweet Potato Soft Rot; Pharmaceutical Prevention and Control; Green Control

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1 Introduction

1.1 Current Situation of Sweet Potato Industry

1.1.1 Sweet Potato Overview

Sweet potatoes, have in recent years become a trending research topic because of their special nutritional and functional properties. Potato is an annual or perennial vine, the bioactive proteins, flavonoids, carbohydrates, carotenoids, anthocyanins, phenolic acids and minerals which are different nutrients found in the leaves and roots of sweet potato[1].Root tubers are mostly used as food, feed and industrial raw materials in most parts of the world were sweet potatoes are grown. As an important and reliable food source, sweet potato can be used for commercial production and also can be grown by self-sufficient and small-scale farmers. A number of different health benefits, such as antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, cardioprotective, antimicrobial, anti-obesity and prevention of vitamin A malnutrition have been reported because of the ingestion of different parts of sweet potato. Compositional analysis showed that the orange-fleshed sweet potato varieties are superior source of β-carotene while the purple-fleshed sweet potato varieties have the excellent levels of anthocyanins. Whereas, the white-fleshed sweet potato varieties nearly contain no β-carotene. The dietary intakes of sweet potatoes are strongly recommended for their undeniable health-promoting properties. They also offer promissing application in food industries for novel value-added food products and that products prepared with or from sweet potato are compiled[1].

Sweet potato belonging to the family of Convolvulaceae, is found to be grown in more than 100 countries in tropical, subtropical and temperate climates[2]. It ranks as the world's seventh most important crop, with an approximated annual production of 110 million metric tons[3]. It is one of the major staple food in Africa, Asia, the Caribbean and South America. In South Africa, sweet potato is a popular household food security and traditional crop which was introduced to South Africa around the time of the colonization of the Cape of Good Hope[4]. Orange-fleshed sweet potato which contains high levels of provitamin A is of particular importance, as of vitamin A deficiency is a national public health problem[5]. β-carotene–rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. The crop is adaptable and can withstand high temperatures, low fertility soil and drought. Sweet potato is a short-season crop which reliably provides food on marginal and degraded soils with little labor and few or no inputs from outside the farm. Increasing recognition of the great potential of sweet potato as a crop for fighting malnutrition and food security has resulted in intensive research efforts in recent years to improve its production and consumption[6]. The sweet potato belonging to the the morning glory family, *Convolvulaceae*, and recent results of molecular marker studies suggest that Central America is the center of origin of sweet potato[7]. Sweet potato has a very high genetic variability and thousands of accessions of sweet potato exist in germplasm collections. More than 8000 accessions, cultivars and breeding lines of sweet potato and nearly 26,000 accessions of other Ipomoea species are maintained in 83 gene banks world-wide[8]. The

characterization of germplasm diversity and the genetic relationships among cultivars, genotypes and breeding lines are critical in crop improvement program. Some of the researchers believed that sweet potato originated in Mexico and tropical America from Colombia, Ecuador to Peru. When Columbus first visited the Queen of Spain, he presented her with sweet potatoes brought back from the New World. At the beginning of the 16th century, sweet potatoes were widely cultivated in Spain. Spanish sailors carried it to Manila and Molucca in the Philippines, where it spread throughout Asia[9]. Sweet potato was introduced into China through several channels, and it was around the end of the 16th century[10]. There are relevant records in Min Shu of the Ming Dynasty, Fu Shu of Rural Administration of The Ming Dynasty, Fu Shu of Min Zheng of the Qing Dynasty and Fu Zhi of Fuzhou. Chen Shiyuan of the Qing Dynasty cited the Record of The Gathering of Min Hou He zhi in his Biography of Golden Potato[11]. During the Reign of Wanli in the Ming Dynasty, Chen Zhenlong, a min man, traded his land, got the vine seedlings and grew them into China. Mid - Fujian drought hunger. Zhenlongzi Jinglun Bai, when the governor Jin Xuecheng ordered him to try to plant it, it yielded a great deal, which could be filled with half of the grain. The sterile field has been proof against seeding everywhere. He then said, "I got it from the Old Chinese name yams. It is planted with gold, so it is also called golden potato." According to further information, Chen Shiyuan, the 6th grandson of Chen Zhenlong, and his son Chen Yun, successively planted sweet potato in Yinzhou (Ningbo, Zhejiang), Jiaozhou, Qingzhou (Qingdao, Yidu, Shandong province) and Yuzhou (Zhuxian Town, Henan Province), and gradually spread throughout Zhejiang.

The above historical facts prove that sweet potato was introduced from Nanyang to Fujian and Guangdong in the late 16th century, and then spread to the Yangtze River, Yellow River basin and Taiwan province. According to some research, China has the world's largest acreage and total output of sweet potatoes[12]. The main producing areas of sweet potato in the world are distributed south of 40° N. Asia had the largest cultivated area, followed by Africa, and then America was the third. In 1985, the cultivated area of sweet potato was 800.3 million hectares, and the total output was 111.438 million tons. Sweet potato is widely distributed in China, with Huaihai Plain, Yangtze River basin and southeast coastal provinces most[13]. The whole country is divided into 5 potato areas: Northern spring potato areas. Including Liaoning, Jilin, Hebei, Northern Shaanxi and other places, the region is short frost-free period, low temperature approach early, planted more spring potato. The potato region in spring and summer of Huanghuai Basin[14].sweet potato belongs to the warm temperate climate of monsoon. It is more suitable to grow potatoes in spring and summer. The planting area accounts for about 40% of the total area of the country. Summer potato is grown in the Yangtze River Basin area, the whole Yangtze River basin except Qinghai and northwest Sichuan plateau[15]. Southern summer and autumn potato area. North of the Tropic of Cancer, south of the Yangtze River basin, in addition to planting summer potato, some areas also grow autumn potato. Southern autumn and winter potato region. The coastal land to the south of the Tropic of Capricornus and The islands such as Taiwan belong to the humid tropical climate, high temperature in summer and small temperature difference between day and night, mainly planting autumn and winter potato. The cropping system is different, it varies from region to region in China[16].

Sweet potato an annual or perennial vine, root tuber that can be used as food, feed and industrial raw material and an important and reliable food source has severeal uses such as it can be used for commercial production, grown by self-sufficient and small-scale farmers etc. It has a number of different health benefits, such as antioxidant, cardio-protective, anti-inflammatory, anti-cancer, anti-diabetic, antimicrobial, anti-obesity and prevention of vitamin A malnutrition as described in the above paragraphs.

Sweet potato it being susceptible to a number of diseases, it is therefore important to research on these diseases that affect them and find the effective way to prevent and control the disease.

1.1.2 Sweet Potato Production

Sweet potato is the favorite commodity in some regions of the world. This tuberous plant has a sweet taste and many people seem to like the taste[17]. Sweet potato *(Ipomoea batatas L.)* belongs to the family *Convolvulaceae* and is one of the popular root crops consumed in the Caribbean. The sweet potato tuber

can be used as a staple and is an excellent source of beta-carotene, vitamins, iron, calcium, magnesium, manganese and potassium which are essential for good health[18]. It is also a great source of dietary fibre especially when eaten with the peel. Sweet potato must be grown in full sunlight for maximum yields. A moderately deep, fine sandy loam soil with adequate drainage is recommended for production of good quality tubers[19]. It should not be planted on areas prone to flooding since excess soil moisture may promote tuber rot. TT: Ag Ext 14:01 A soil pH of 5.6 - 6.5 is ideal. A soil test is recommended to determine the soil pH and the amount of limestone and fertilizer needed for optimum crop growth[20].
Based on FAO Statistical Database, there are Top World's Biggest Sweet Potato-Producing Countries. Historically, sweet potatoes are native to the tropical regions of the Americas.

Year	Production (Million metric ton)
2010	93.67
2011	94.21
2012	90.85
2013	90.33
2014	93.63
2015	91.58
2016	90.63
2017	92.52
2018	91.48
2019	91.49
2020	89.49

Table 1.1 Top World's Biggest Sweet Potato-Producing Countries in 2020

No.	Country	Production (Million metric ton)
1	China	48.95
2	Malawi	6.92
3	Tanzania	4.44
4	Nigeria	3.87
5	Angola	1.73
6	Ethiopia	1.6
7	United States	1.56
8	Uganda	1.54
9	Indonesia	1.49

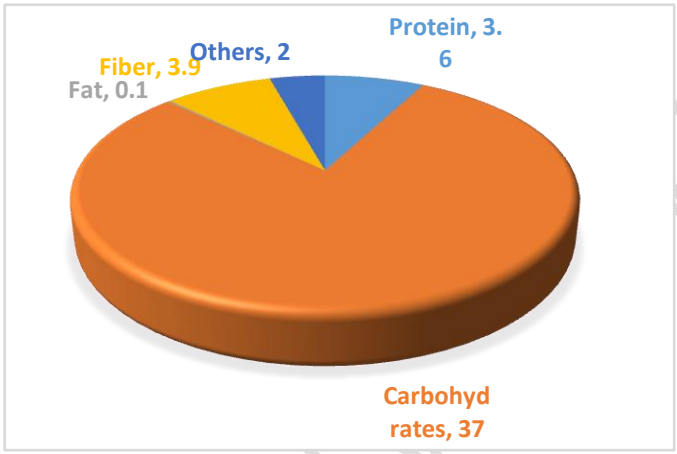
(Source: FAO Statistical Database)
The above table shows the statistical database of the top world's biggest sweet potato-producing countries in the year 2020, China is the highest producer with a record of 48.95 million metric tons.

Table 1.2 Sweet potato production worldwide from 2010 to 2020

(Source: FAO Statistical Database)
The above table shows the statistical database of the sweet potato production worldwide from 2010 to 2020, production was the highest in 2011 with a record of 94.21 million metric ton

1.1.3 Sweet Potato Nutritional fact and Health Benefits

Sweet potato have colorful, health-boosting nutrients. It is a low-calorie, fat-free, nutrient-dense source of healthy carbohydrates, fiber, and many vitamins and minerals including vitamin A, potassium, and vitamin C. Sweet potatoes are rich in antioxidants that have been studied for cancer prevention and treatment. Purple sweet potatoes, in particular, are high in anthocyanins, which appear to promote apoptosis of cancer cells. The anthocyanins in sweet potatoes are also associated with anti-inflammatory effects that reduce the risk of heart disease. Some certain pro-inflammatory cytokines appear to be suppressed in response to purple sweet potato extract. Furthermore, the fiber in any vegetable reduces cholesterol, while the high potassium levels of sweet potatoes keep blood pressure down.



(Source: Authors own compilation with data obtained from USDA)

Figure 1. Sweet Potato Nutrients Content in grams

The above figure shows nutrients content of one large sweet potato (180g). 3.6g of protein, 37g of carbohydrates, 0.2g of fat and others 2g. Sweet potato is an excellent source of vitamin C, vitamin A, potassium and also provides 162 calories.

1.2 Sweet Potato Soft Rot

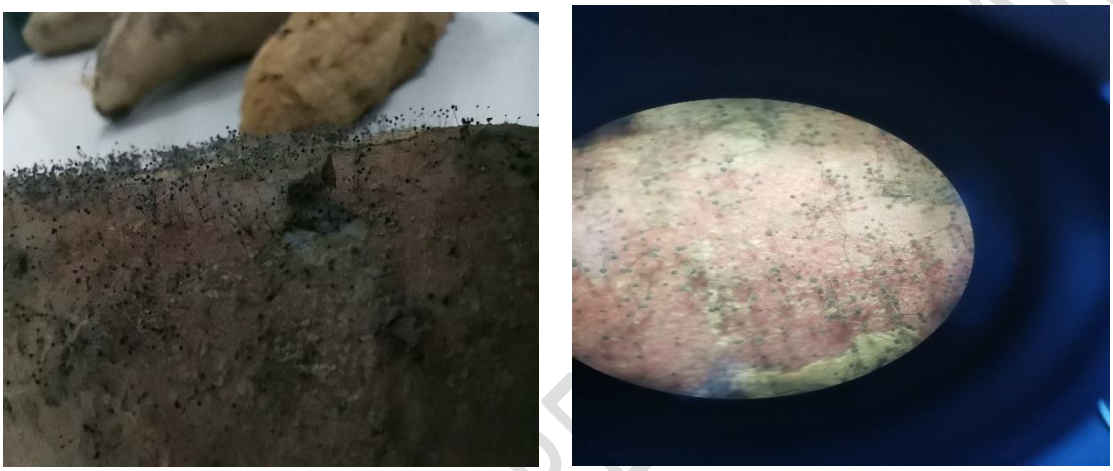
1.2.1 General Overview

Sweet potatoes are susceptible to a number of diseases, among these is fungal soft rot of sweet potato. Sweet potato soft rot is caused by the necrotrophic, Zygomycete fungus Rhizopus stolonifer[21]. The production of sweet potatoes is accompanied by severe diseases caused by fungus R. stolonifer leading to enomrous losses in yield and quality worldwide[21]. It is one of the most common disease of sweet potatoe soft rot. Rotting of tubers by fungal soft rot during storage varies from 31.3% to 36.8%. R. stolonifer is a ubiquitous fungus that also causes postharvest soft rot on several fruits and vegetables, most notably sweetpotato and stone-fruits[22]. This soft rot disease has an economic importance storage problem because of their pathogenicity to a wide range of crops from which potatoes are the most important. Rhizopus soft rot typically appears during postharvest handling, transporting and is rarely seen in the field. Symptoms usually originate at a wounded area in the sweet potato and consist of a soft, watery rot that progresses quickly under suitable conditions and can result in full decay of an infected root in few days mostly in three days[23]. White to gray fungal mycelium producing black sporangia are often observed growing on decayed roots. One of the most important methods to control rhizopus soft rot disease in sweet potato all over the world is the chemical methods using antibiotics and fungicides[24].

1.2.2 Symptoms and Signs

Rhizopus soft rot typically appears during handling and transporting at postharvest time and is rarely

observed in the field. Symptoms originate at a wounded area in the sweet potato and consist of a soft, watery rot that progresses quickly under favorable conditions[25]. *R. stolonifer* requires wounds in the sweet potato root, specifically for bruised wounds, for the disease to initiate. Disease progression and sporulation are then significantly influenced by storage conditions. While disease can occur over a wide range of temperatures, relative temperatures of 25-29°C favor disease progression. However higher humidities significantly raise sporulation of *R. stolonifer*, leading to increased secondary inoculum. Initial inoculum levels also have little impact on disease progression but do support higher amounts of secondary inoculum[26]. During storage, some roots may appear untouched by the disease until they are cut into wherein decay becomes evident. The infected roots are streaked with black and become soft, moist and rotten. Below shows the pictures to describe the above information on the symptoms of sweet potato soft rot disease.



A B

Figure 2: Sweet Potato Soft Rot *Rhizopus stolonifer*

The above figures shows sweet potato soft rot, figure A shows the outer surface layer of the soft rot disease and figure B shows a more detailed view.

1.3 *Rizhopus stonolifer*

1.3.1 General Characteristics

Rhizopus stolonifer is a cosmopolitan phytopathogenic fungus which belongs to the Mucoraceae family, it is able to grow and develop in a wide variety of environments[27]. It is a fast growing specie, which favors the colonization and decomposition of stored food or agricultural products. This specie is one of the most common fungi, distributed worldwide, although it is more frequent in tropical and subtropical areas. However it is a saprophytic organism that intervenes in the colonization of the soil substrate and acts as a parasite, invading decomposing plant tissues. Commonly known as "black bread mold", it is characterized by a dense branched mycelium, composed of three types of hyphae, namely, stolons, sporangiophores and rhizoids[28]. Sporangia produce numerous multinucleated spores that are involved in asexual reproduction, as opposed to sexual reproduction, which requires compatible but physiologically different mycelia. At the industrial level the fungi of the genus rhizopus (*R. stolonifer*), they are used for the production of ethyl alcohol product of fermentation[29]. In addition, this species is used commercially to obtain high purity lactic acid and fumaric acid used in the chemical, food and pharmaceutical industries[30].

1.3.2 Distribution and Habitat

The zygomycota fungus *Rhizopus stolonifer* is one of the most common mucorales, since it is broadly distributed throughout the world. Indeed, it is one of the first fungi to appear on stale bread, rotting fruits and vegetables, as an organism in perishable foods. It thrives effectively in a temperature range between 12 and 32º C, the optimum growth temperature being 25º C. Nevertheless, its spores are rare in fresh air environments, but they are abundant in humid environments and around decomposing biological products[31]. They are usually located on moist soil, in compost or manure, on disaggregated plant material or in accumulated dust. Likewise, on wood pulp, bird nests, honeycombs, or on various seeds and fruits[32].

Indeed, this species is able to colonizing a wide variety of natural substrates, as it adapts to different concentrations of vital nutrients. In fact, nitrogen and carbon can simply be used or in combination with different nutrients. At the laboratory level, *R. stolonifer* is grown on various culture media, including those containing amino compounds and ammonium salts[33]. However, it does not grow on media with a high nitrate content, such as Czapek Dox Agar, which has nitrate as the only source of nitrogen. In cultivation, the zygospores of *R. stolonifer,* they germinate after 8-20 days at an average temperature of 21º C. However, the spores of this fungus require a period of rest prior to the germination process and mycelial development[34].

1.3.3 Life Cycle

The *Rhizopus stolonifer* reproduces sexually or asexually. In fact, it is a heterothalic organism, which for its sexual reproduction requires the mating of two thalli of different charges to create a sexed spore[35].

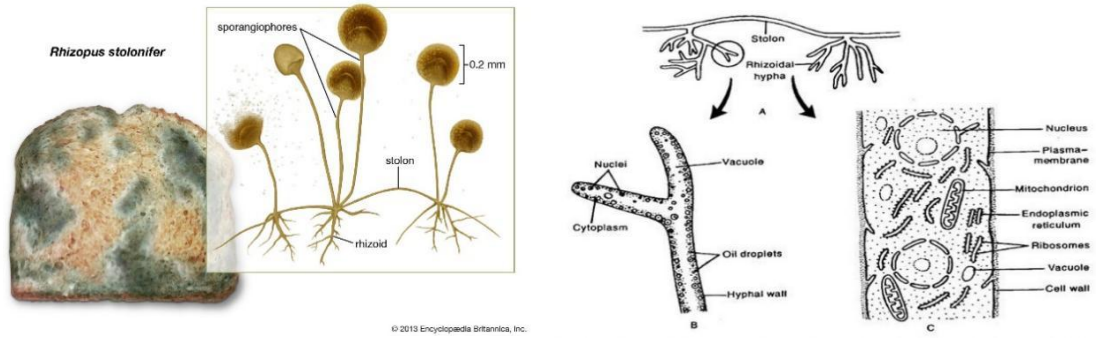
1.3.3.1 Sexual Reproduction

Sexual reproduction occurs under unfavorable conditions with the mating that have compatible strains, ultimately resulting in the zygospore[36]. Indeed, sporangiophores harbor mating strains of the positive "+" or negative "-" type which facilitates their union. In *Rhizopus stolonifer* Sexual reproduction begins when the progametangia or specialized hyphae of two strains of different sign fuse[37]. This attraction between dissimilar progametangia transpires with the intervention of diffused hormones in the form of gases. Next, two gametangia or apical cells arise, each containing abundant "+" nuclei and the other containing "-" nuclei. The gametangia fuse, causing the union of numerous pairs of nuclei "+" and "-", forming diploid nuclei. In this way, the zygosporangium is produced, a product of multinucleated cells with a firm, pigmented and rough cell wall, which contains a single zygospore[38]. Under favorable environmental conditions, diploid nuclei undergo the meiosis process just before germination occurs. During germination, the zygosporangium wall breaks, releasing the zygospore giving rise to the sporangiophore[38]. At the end of the sporangiophore a sporangium is located that will give rise to the spores, which once germinated will form a "+" or "-" mycelium. The mushroom *R. stolonifer*, it behaves like a haploid organism during most of the sexual reproduction cycle. In addition, the mycelium is made up of numerous branched hyphae that fulfill the function of support and nutrition of the fungus[39].

1.3.3.2 Asexual Reproduction

The asexual cycle occurs under suitable or favorable conditions when the production of sporangiophores begins from sporangia containing sexually spores that are compatible. The form of

dispersal of the spores is favored by the wind, as the sporangia mature, their thin wall disintegrates releasing the spores[40]. Aerial hyphae are sourced from the internodes and grow to a certain height. The nuclei and cytoplasm then cluster toward the apical end, promoting the growth of the apex of the aerial hyphae. This area grows globularly giving rise to a rounded sporangium, formed by the sporoplasm and the columella[41]. The nuclei of the sporoplasm undergo a rapid division until they change into spongiospores. As these structures mature, the columella disintegrates, releasing large amounts of sporangiospores into the atmosphere. In a suitable substrate and under favorable temperature and humidity conditions these spores germinate. In this manner, a new complex of hyphae is formed that will expand to continue the cycle. Under adverse conditions, septa are produced in the intercalary mycelium, giving rise to a plump spore called chlamydiospora[42].



X Y

Figure 3. Structure and Morphology of *Rhizopus Stolonifer*

(Source: Rhizhopus stolonifer/ fungus/Britannica)

The above picture X shows the structure of *Rhizopus Stolonifer* and picture Y shows the morphology of *Rhizopus stolonifer*. The letters in picture Y represent A-Vegetative mycelium, B-Portion of hypha under light microscope, C-Portion of hypha under electron microscope.

1.4 Fungicides

There are several different kinds of control agents or fungicides used in agriculture, however in this research, only some among the several kinds of fungicides were used. Namely; Tebuconazole, fludioxonil, Procymidone, Fluazinam, Pyraclostrobin, Prochloraz, Kresoxim-methyl, Azoxystrobin, Prothionazole, Octreotide, Boscalid, Difenoconazole, Thifluzamide, Carbendazim, Pyraclostrobin, Triadimenol.

1.4.1 Tebuconazole

Tebuconazole (Chemical formula $C_{16}H_{22}ClN_3O$, molecular weight 307, CAS accession number 107534-96-3, melting point 102.4 °C, density 1.25 g / cm ³ Colorless crystal in appearance) is an active ingredient from the triazole family of fungicides. It is a systemic fungicide and delivers both curative and preventative control of diseased plants. Tebuconazole is used in a number of different popular fungicide products to control fungi, bacteria, and viruses affecting various plants[43]. Some of the common fungal and disease problems Tebuconazole is known to treat are rust fungus, leaf spot, sheath blight, and anthracnose. Tebuconazole is a fungicide that is known as a DMI (demethylation sinhibiting fungicide),

this implies that tebuconazole works by affecting the cell walls of fungi by suppressing spore germination and fungus growth[44]. It also tempers with the production of ergosterol—a molecule essential to the formation of fungus. As a result, the formation of fungus is slowed and eventually stopped. Because of this unique mode of action Tebuconazole is considered to be fungistatic or growth-inhibiting rather than fungicidal or fungus killing[45]. Tebuconazole is a flexible fungicide that can be used for both profitable and preventative fungus control. It works systemically, absorbing into the target plant to protect it against diseases, prevent further spread or can eliminate the disease entirely depending on the severity level[46]. It is helpful against diseases but only at the proper rates. If too much active ingredient is absorbed into the plant, it can cause phytotoxicity, poisoning the plant. Tebuconazole has a residual effect of up to 30 days after application[47]. Spray monthly for preventative control of listed diseases, but do not apply more than 16 oz. per 1000 sq. ft. per calendar year. Depending on the targeted disease, there should see a noticeable improvement in your plant's health in one to three weeks.

1.4.2 Fludioxonil

Fludioxinil (CAS accession number 131341-86-1, molecular weight 248.15, density 1.6±0.1 g/cm3, boiling point 420.4±45.0 °C at 760 mmHg, Molecular formula $C_{12}H_6F_2N_2O_2$ melting point 199.4°, flash point 208.0±28.7 °C). Fludioxonil is a member of the class of benzodioxoles that is 2,2-difluoro-1,3-benzodioxole substituted at position 4 by a 3-cyanopyrrol-4-yl group[48]. A fungicide seed treatment for control of a wide-range of diseases including Fusarium, Rhizoctonia and Alternaria. It has a role as an androgen antagonist, an estrogen receptor agonist and an antifungal agrochemical. It is a member of benzodioxoles, also a member of pyrroles, nitrile and an organofluorine compound[49]. Fludioxonil is produced by reaction of 4-amino-2,2-difluorobenzodioxide in HCl-containing acetic acid with sodium nitrite at 0 °C, then followed by an addition of ethylenenitrile, methyl ethyl ketone, and a solution of copper(I) chloride in hydrochloric acid[50]. The product is extracted with dichloromethane and the thus-obtained solution heated to reflux in the presence of triethylamine to give 2,3-(difluoromethylenedioxy) cinnamic acid nit. Fluazinam is a wide spectrum contact fungicide that can be applied as a foliar spray or soil treatment. It is effective against a number of pathogenic fungi that can cause the following diseases: gray mold and downy mildew in grapes, melanose and mites in citrus, scab and Alternaria blotch in apples, clubroot in crucifers, Sclerotinia blight in peanuts, white root rot and violet root rot on fruit trees[51]. It's best known however due to the fact that it protects against foliar blight, tuber blight and sclerotinia rot in potatoes caused by the fungus Phytophthora infestans. The effect on potatoes is unparalleled. Fluazinam is very effective against both spore germination and spore growth, thereby giving it protective action with a good residual effect[52]. It has good rain fastness. The mode of action of fluazinam is due to the disruption of energy production in the fungus at multiple sites therefore preventing resistance. Floozee is the Agchem Access branded form of fluazinam, sold as 500 g/l fluazinam as a suspension concentrate. Rile, which is treated with p-toluenesulfonylmethyl isocyanide and potassium tert-butoxide in THF[53].

1.4.3 Procymidone

Procymidone (CAS number 32809-16-8, Molecular formula C13H11Cl2NO2, molar mass 284.138g/mol, molecular weight 284.13, melting point 166.0°C). Procymidone and vinclozolin are dicarboximide fungicides commonly used for controlling diseases, such as blights, rots and molds in vineyards and on certain fruits and vegetables[54]. Workers can be exposed to them during handling activities such as mixing, loading, applying and flagging, or by re-entering treated sites. Procymidone, on the contrary, exhibits almost identical affinity for the AR than the antiandrogenic drug flutamide, while its metabolites shows varying but lower levels of affinity[55]. When administered during sexual development in a murine model, vinclozolin and procymidone, it produces an undervirilization and feminization of male pups, attested by a reduction in anogenital distance, retained nipples, hypospadias, a

blind vaginal pouch, reduced to absent accessory glands, and a delay in pubertal maturation; such malformations lead to a greater rate of infertility at adult age. Vinclozolin has also been shown to promote transgenerational inheritance of reduced male fertility: after a transient embryonic exposure at a critical time during gonadal sex determination, an adult testis phenotype of decreased spermatogenic capacity and male infertility was found to be transgenerational, and appeared to be associated with altered primordial germ cell epigenetic programming via noncoding RNAs and alterations in gene expression[56]. In spite of these concordant data in laboratory models, no epidemiological study has specifically looked into the antiandrogenic effects of vinclozolin or procymidone in men. Both vinclozolin and procymidone have now been phased-out for most domestic food uses[57]. The imidazole fungicide prochloraz was introduced in the late 1970s for horticulture and agriculture purposes in Europe, Asia, Australia, and South America[58]. It is still currently in use on wheat, barley, mushrooms, cherries, turf on golf courses, and in flower production. The action of imidazoles used as fungicides or antimycotic drugs (e.g., ketoconazole) is based on the inhibition of the cytochrome P450-dependent 14 alpha-demethylase activity required in the conversion of lanosterol to ergosterol, an essential component of fungal cell membranes. The molecular basis of this inhibition is the presence of an imidazole moiety that interacts strongly with the iron atom of cytochrome P450[58]. However, this binding is fairly unspecific, and prochloraz also inhibits the activities of a wide spectrum of other cytochrome P450-dependent enzymes, including key enzymes involved in steroidogenesis such as CYP17A1. Prochloraz is also an AR antagonist, and even though some research suggests that its dominant mechanism of action is the inhibition of testosterone production, its AR antagonistic properties are likely to be magnified by decreased circulating testosterone levels[59]. In murine models, gestational exposure to prochloraz produces a significant decrease in testosterone levels in male fetuses and smaller anogenital distance, nipple retention, and hypospadias in the male offspring. Peripubertal exposure to prochloraz also delays pubertal development. A brief exposure at adult age does not significantly affect the adult male reproductive function. As of today, there are no epidemiological studies available on the effects of prochloraz in men reproductive function.

1.4.4 Fluazinam

Fluazinam (CAS number 79622-59-6, Chemical formula C13H4Cl2F6N4O4, density1.8±0.1 g/cm3, melting point116 ℃ , boiling point 376.1±42.0℃ , Solubility in water1.76 mg/L). Fluazinam is a wide spectrum contact fungicide that can be applied as a foliar spray or soil treatment[60]. It is effective against a number of pathogenic fungi that can cause the following diseases: gray mold and downy mildew in grapes, melanose and mites in citrus, scab and Alternaria blotch in apples, white root rot and violet root rot on fruit trees, clubroot in crucifers, Sclerotinia blight in peanuts. It's best known however due to the fact that it protects against foliar blight, tuber blight and sclerotinia rot in potatoes caused by the fungus Phytophthora infestans. The effect on potatoes is unparalleled[61]. Fluazinam is effective against both spore germination and spore growth, thereby giving it protective action with a good residual effect. It also has good rainfastness. The mode of action of fluazinam is due to the disruption of energy production in the fungus at multiple sites, therefore preventing resistance[62]. Floozee is the Agchem Access branded form of fluazinam, sold as 500 g/l fluazinam as a suspension concentrate.

1.4.5 Pyraclostrobin

Pyraclostrobin (Molecular formula C19H18ClN3O4, CAS number 175013-18-0, molecular weight 387.81700, melting point 63.7-65.2 ℃) is a carbamate ester that is the methyl ester of [2-(methyl)phenyl]methoxycarbamic acid. A fungicide used to control major plant pathogens including Septoria tritici, Puccinia spp. and Pyrenophora teres[63, 64]. It has a role as a mitochondrial cytochrome-bc1 complex inhibitor, a xenobiotic, an environmental contaminant and an antifungal agrochemical. It is a member of pyrazoles, a carbamate ester, and an aromatic ether, a member of monochlorobenzenes, a

methoxycarbanilate strobilurin antifungal agent and a carbanilate fungicide. Pyraclostrobin is also a member of the strobilurin group of fungicides[61, 65]. The strobilurin fungicides act through inhibition of mitochondrial respiration by blocking electron transfer within the respiratory chain, which in turn causes important cellular biochemical processes to be severely disrupted, and results in cessation of fungal growth[59, 66].

1.4.6 Carbendazim

Carbendazim (Molecular formula C9H9N3O2, Molecular weight 191.19, CAS number 10605-21-7, melting point 576 to 585 °F) is a wide-spectrum benzimidazole antifungal with potential antimitotic and antineoplastic activities. Although the exact mechanism of action is unclear, carbendazim appears to binds to an unspecified site on tubulin and suppresses microtubule assembly dynamic. This results in cell cycle arrest at the G2/M phase and an induction of apoptosis. Carbendazim is a member of the class of benzimidazoles that is 2-aminobenzimidazole in which the primary amino group is substituted by a methoxycarbonyl group. A fungicide, carbendazim controls Ascomycetes, Fungi Imperfecti, and Basidiomycetes on a wide variety of crops, including bananas, cereals, cotton, fruits, grapes, mushrooms, ornamentals, peanuts, sugarbeet, soybeans, tobacco, and vegetables. It has a role as an antinematodal drug, a metabolite, a microtubule-destabilising agent and an antifungal agrochemical. It is a carbamate ester, a member of benzimidazoles, a benzimidazole fungicide and a benzimidazolylcarbamate fungicide. It derives from a 2-aminobenzimidazole. Carbendazim appears as light gray or beige powder[67, 68]. Carbendazim, or MBC, was introduced as a commercial fungicide in 1972 and has through 1972-1973 obtained registration and approval for specified pre-harvest treatments in a number of countries in temperate, subtropical and tropical regions. It is available as wettable powder formulations or as dispersions containing 60 and 20% active ingredient, respectively. Accordingly, the antifungal effects of carbendazim are described as practically similar to the two mentioned chemicals, i.e. it is a broad-spectrum, systemic fungicide which is active against moulds, rots and blight. It is claimed effective against apple scab, powdery mildew, botrytis and Penicillim induce decay of citrus fruits[69].

1.4.7 Azoxystrobin

Azoxystrobin (Molecular formula C22H17N3O5, molecular weight 403.4, CAS number 131860-33-8, melting point 116.0 °C) is a wide spectrum fungicide mostly used for protecting plants and food crops from foliar and soil-borne fungal diseases. It is currently the only fungicide capable of protecting against all four major groups of fungal diseases. Azoxystrobin is an aryloxypyrimidine having a 4,6-diphenoxypyrimidine skeleton in which one of the phenyl rings is cyano-substituted at C-2 and the other carries a 2-methoxy-1-(methoxycarbonyl)vinyl substituent, also at C-2. An inhibitor of mitochondrial respiration by blocking electron transfer between cytochromes b and c1, it is used broadly as a fungicide in agriculture. It has a role as a mitochondrial cytochrome-bc1 complex inhibitor, a xenobiotic, an environmental contaminant, an antifungal agrochemical and a quinone outside inhibitor. It is a nitrile, a aryloxypyrimidine, a enoate ester, a enol ether, a methyl ester and a methoxyacrylate strobilurin antifungal agent. Azoxystrobin is a methoxyacrylate analog and a strobilurin fungicide[70]. Azoxystrobin is a systemic fungicide. It slowly works its way up through the plant roots and into all the parts of the plant, inhibiting mitochondrial respiration in fungi. This puts a stop to spore germination and fungus growth. Azoxystrobin is used broadly in agriculture, particularly in wheat farming. Its use is limited in the United States. It has low toxicity for mammals, birds, bees, insects, and earthworms. Toxicity for freshwater fish is high. Azoxystrobin is also very toxic to Macintosh apple trees and may damage crabapples.

1.4.8 Prothionazole

Prothionazole (Molecular formula $C_{14}H_{15}C_{l2}N_3OS$, molecular weight 344.3, CAS number

178928-70-6, melting point 139.1-144.5 ℃, boiling point 587 ℃) 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-1,2,4-triazole-3-thione belongs to the class of triazoles that is 1,2,4-triazole-3-thione substituted at position 2 by a 2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl group. Prothionazole is a member of monochlorobenzenes, a member of triazoles, a tertiary alcohol, a member of cyclopropanes and a thiocarbonyl compound. Is a wide-spectrum systemic fungicide formed by Bayer CropScience for the control of diseases caused by ascomycetes, basidiomycetes, and deuteromycetes[71]. Prothioconazole may be applied alone or as a tank mix with other fungicides, insecticides, or herbicides. Application through any type of irrigation system is prohibited. EPA and the Pest Management Regulatory Agency (PMRA) of Canada jointly reviewed prothioconazole. The crops proposed for joint review include barley, canola, chickpeas, the oilseed crop group, the dried shell and bean subgroup, lentils and wheat. Uses on peanuts were proposed for the U.S. only. Prothioconazole is formulated as a 4 lb/gal suspension concentrate (equivalent to a flowable concentrate; FlC) formulation (Proline® 480 SC Fungicide, 41% active ingredient). The product is applied as broadcast post emergence foliar or soil sprays (application to soil for peanuts only) using ground or aerial equipment at 0.088-0.178 lb ai/A/application (0.100-0.200 kg ai/ha/application). The proposed maximum seasonal rates range 0.285-0.713 lb ai/A (0.320- 0.800 kg ai/ha), and the proposed retreatment intervals are 5-21 days. The PHIs range from 7 days for dried shelled peas and beans to 36 days for oilseed crops.

1.4.9 Octreotide

Octreotide (Molecular formula $C_{49}H_{66}N_{10}O_{10}S_2$, molecular weight 1019.2, CAS number 83150-76-9, melting point 153-156°C, boiling point 1447°C) is a synthetic long-acting cyclic octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. Octreotide is a more potent inhibitor of growth hormone, glucagon, and insulin than somatostatin. Similar to somatostatin, this agent also suppresses the luteinizing hormone response to gonadotropin-releasing hormone, decreases splanchnic blood flow, and inhibits the release of serotonin, gastrin, vasoactive intestinal peptide (VIP), secretin, motilin, pancreatic polypeptide, and thyroid stimulating hormone[72]. Octreotide is a synthetic somatostatin analogue that resembles the native polypeptide in its activity in suppressing stages and activity of growth hormone, insulin, glucagon and many other gastrointestinal peptides. Because its half-life is longer than somatostatin, octreotide can be used clinically to treat neuroendocrine tumors that secrete excessive amounts of growth hormone (acromegaly) or other active hormones or neuropeptides. Octreotide has many side effects including suppression of gall bladder contractility and bile production, and maintenance therapy can cause cholelithiasis, pancreatitis as well as clinically apparent liver injury. Octreotide is a long-acting drug with pharmacologic activities that mimic those of the natural hormone, somatostatin, which inhibits the secretion of growth hormone[73]. Additionally, it is used for the treatment of acromegaly and symptoms arising from various tumors, including carcinoid tumors and vasoactive intestinal tumors (VIPomas).

1.4.10 Boscalid

Boscalid (Molecular formula of $C_{18}H_{12}Cl_2N_2O$, molecular weight 343.2, CAS number 188425-85-6, melting point 142.8 to 143.8 °C) is a pyridinecarboxamide derived by formal condensation of the carboxy group of 2-chloronicotinic acid with the amino group of 4'-chlorobiphenyl-2-amine. A fungicide active against a wide range of fungal pathogens including Botrytis spp., Alternaria spp. and Sclerotinia spp. for use on a wide range of crops including vegetables, fruits and ornamentals. It has a role as an EC 1.3.5.1 [succinate dehydrogenase (quinone)] inhibitor, an environmental contaminant, a xenobiotic and an antifungal agrochemical. It belongs to a group of biphenyls, a pyridinecarboxamide, a member of monochlorobenzenes and an anilide fungicide[74]. It is obtained from a nicotinic acid. Boscalid is an agricultural fungicide active against a broad range of fungal pathogens such as alternaria blight, botrytis,

powdery mildew, purple blotch, rust, leaf spot, target spot, brown spot, white mold, gray mold, stem rot and sclerotinia. It is applied to numerous variety of crops and vegetables including beans, brassicas, onions, garlic, shallots, peas, carrots, turnips; soybeans; fruits including grapes, apples, pears, strawberries; and tree nuts. Boscalid is also used as a seed treatment and protectant. The pure compound is characterized as a white crystalline powder. It is often supplied as soluble granules which are mixed with water and applied as a spray[75].

1.4.11 Difenoconazole

Difenoconazole (Molecular formula $C_{19}H_{17}Cl_2N_3O_3$, molecular weight 406.3, boiling point 100.8 °C (3.7 mPa), CAS number 119446-68-3, melting point 76.0 °C) it belongs to the class of dioxolanes that is 1, 3-dioxolane substituted at position 2 by 2-chloro-4-(4-chlorophenoxy) phenyl and 1, 2, 4-triazol-1-ylmethyl groups. A broad spectrum fungicide with novel wide-range activity used as a spray or seed treatment. It is moderately toxic to humans, mammals, birds and most aquatic organisms. It has a role as an environmental contaminant, a xenobiotic, an EC 1.14.13.70 (sterol 14alpha-demethylase) inhibitor and an antifungal agrochemical. It is an aromatic ether, a dioxolane, a member of triazoles, a cyclic ketal, a conazole fungicide and a triazole fungicide. It is a wide-spectrum fungicide mostly used for disease control in many fruits, vegetables, cereals and other field crops. It has preventive and curative action. Difenoconazole acts by inhibition of demethylation during ergosterol synthesis[76].

It is applied by foliar spray or seed treatment and controls a wide-spectrum of foliar, seed and soil-borne diseases caused by Ascomycetes, Basidiomycetes and Deuteromycetes on a variety of crops. Difenoconazole was evaluated for the first time by JMPR 2007. The 2007 Meeting established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw and an acute reference dose (ARfD) of 0.3 mg/kg bw. In 2007, 2010 and 2013, the JMPR evaluated the compound for residues and recommended a number of maximum residue levels. Difenoconazole was listed by the 46th session of CCPR (2014) for evaluation for additional MRLs. The current Meeting received from the manufacturer additional analytical methods, processing data from soya beans, oilseed rape and rice, GAP information and residue trial data from uses on strawberry, avocado, soya beans, cotton, peanut, rice and oilseed rape (canola).

1.4.12 Thifluzamide

Thifluzamide (Molecular formula $C_{13}H_6Br_2F_6N_2O_2S$, molecular weight 528.06, melting point 178.0 °C, solubility 3.03e-06 M, CAS number 130000-40-7) is an aromatic amide derived by formal condensation of the carboxy group of 2-methyl-4-(trifluoromethyl)thiazole-5-carboxylic acid with the amino group of 2,6-dibromo-4-(trifluoromethoxy)aniline. Used to control Rhizoctonia spp. diseases on rice, potatoes, maize, grass and other crops. It has a role as an EC 1.3.5.1 [succinate dehydrogenase (quinone)] inhibitor and an antifungal agrochemical. It is an aromatic amide, an aromatic ether, an organofluorine compound, a member of 1, 3-thiazoles, a dibromobenzene and an anilide fungicide. It is a member of the thiazolamide class of bactericides, with strong systemic conductivity and long lasting effect[77]. Thifluzamide is active against pathogenic fungi such as Rhizoctonia, Puccinia spp, Ustilago, Tilletia, Achilles, Rhizoctonia and other pathogenic fungi, especially against Basidiomycetes. Diseases such as sheath blight and wilt have specific effects. Thifluzamide has a good control effect on several kinds of fungal diseases, and can be broadly used in rice, wheat, peanut, cotton, sugar beet, coffee, potato, lawn and other crops. In production, it is commonly used to control sheath blight of rice and wheat. Thifluzamide is suitable for various application methods such as foliar spray, seed treatment and soil treatment[78]. Foliar spray is often used for the prevention and control of sheath blight. Booting stage and before is the main period for the prevention and control of sheath blight. Generally, 240 g/l suspension agent 20-25 ml is used per 667m², and 30-45 liters of water is sprayed. The spraying should be uniform and thoughtful. For the prevention and control of rice sheath blight, due to its long lasting period, it only needs to be applied once during the whole growth period of rice, that is, 30 days before the heading of the

rice, use 15-25 ml of 24% suspending agent per 667m², and water 50- 60 kg spray[79].

1.4.13 Kresoxim-methyl

Kresoxim-methyl (Molecular formula $C_{18}H_{19}NO_4$, molecular weight 313.3, melting point 99.0 ℃, CAS number 143390-89-0, solubility 6.38e-06 M) is a carboxylic ester that is the methyl ester of (2E)-(methoxyimino){2-[(2-methylphenoxy)methyl]phenyl}acetic acid. A fungicide for the control of scab on apples and pears and other fungal diseases on a broad range of crops. It has a role as a mitochondrial cytochrome-bc1 complex inhibitor, an environmental contaminant, a xenobiotic and an antifungal agrochemical. It is an oxime O-ether, an aromatic ether, a methyl ester and a methoxyiminoacetate strobilurin antifungal agent. The technical drug of etheraxyl is a white powder crystal, melting point: 97.2-101.7 ℃, density: 1.258kg/l (20 ℃), vapor pressure: 1.3 * 10-6pa (25 ℃), solubility: 2mg / L (20 ℃). Acute oral LD50: the acute oral LD50 of male and female rats is greater than 5000mg / kg. Acute percutaneous LD50: the acute percutaneous LD50 of both male and female rats was greater than 2000mg / kg. No irritation to rabbit eyes and skin. Ames test, mouse sperm teratogenicity test and mouse micronucleus test were negative. Kresoxim-methyl, is an efficient, wide-spectrum and new fungicide[80]. It has good control effect on Strawberry Powdery mildew, melon powdery mildew, cucumber powdery mildew, pear scab, grape white rot and other diseases. Ether mycoester can inhibit the invasion of pathogenic spores, has good protective activity, and comprehensively and effectively control various fungal diseases of vegetables, fruit trees, flowers and other plants, such as powdery mildew, scab, anthrax, rust, blight and so on.

1.4.14 Pyraclostrobin

Pyraclostrobin (CAS number 175013-18-0, molecular formula $C_{19}H_{18}ClN_3O_4$, molecular weight 387.817, density 1.3±0.1 g/cm3, boiling point 501.1±60.0 ℃ at 760 mmHg, melting point 63.7-65.2°). Pyraclostrobin is a carbamate ester that is the methyl ester of [2-({[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy}methyl)phenyl]methoxycarbamic acid. A fungicide used to control major plant pathogens including *Septoria tritici*, *Puccinia* spp. and *Pyrenophora teres*[81]. It has a role as a mitochondrial cytochrome-bc1 complex inhibitor, a xenobiotic, an environmental contaminant and an antifungal agrochemical. It is a member of pyrazoles, a carbamate ester, and an aromatic ether, a member of monochlorobenzenes, a methoxycarbanilate strobilurin antifungal agent and a carbanilate fungicide. Pyraclostrobin is a fungicide belonging to the group which is collectively known as strobilurins, which inhibit mitochondrial respiration. This leads to a reduction of energy-rich ATP that is available to support a range of vital processes in the fungal cell. Pyraclostrobin is one of the most heavily used fungicides, and has been detected on several variety of produce, suggesting human exposure occurs regularly. Recently, pyraclostrobin exposure has been linked to a variety of toxic effects, including neurodegeneration and triglyceride (TG) accumulation[82].

1.4.15 Triadimenol

Triadimenol (Molecular formula $C_{14}H_{18}ClN_3O_2$, molecular weight 295.76, melting point 124.0 ℃, density 1.22 g/cu cm, solubility 4.06e-04 M, CAS number 55219-65-3). Triadimenol is belongs to the class of triazoles that is 3,3-dimethyl-1-(1,2,4-triazol-1-yl)butane-1,2-diol substituted at position O1 by a 4-chlorophenyl group. A fungicide for cereals, beet and brassicas used to control a wide range of diseases including powdery mildew, rusts, bunts and smuts. It has a role as an EC 1.14.13.70 (sterol 14alpha-demethylase) inhibitor, a xenobiotic metabolite and an antifungal agrochemical. It is an aromatic ether, a member of monochlorobenzenes, a conazole fungicide, a triazole fungicide, a secondary alcohol and a hemiaminal ether. Triadimenol, a metabolite of Triadimefon, is a wide-spectrum chiral triazole fungicide that is formed by reduction of a carbonyl group to the corresponding alcohol[83]. Triadimenol

has the potential to be a potent exosome biogenesis and/or secretion inhibitor.it is a systemic fungicide with protective, curative and eradicant action. Absorbed by the roots and leaves, with ready translocation in young growing tissues, but less ready translocation in older, woody tissues. Triadimenol is used for controlling of powdery mildews, rusts and Rhynchosporium in cereals, and, when applied as a seed treatment, control of bunt, smuts, Typhula spp., seedling blight, leaf stripe, net blotch and other cereal diseases. Also used on vegetables, ornamentals, coffee, hops, vines, fruit, tobacco, sugar cane, bananas and other crops, mainly against powdery mildews, rusts and various leaf spot diseases. Application rates as a spray are in the range 100-250 g/ha for bananas and cereals, 125-250 g/ha protective and 250-500 g/ha eradicative for coffee, 0.0025-0.0125% for grapes, pome and stone fruit and vegetables; application rates as a seed treatment are in the range 20-60 g/100 kg seed for cereals, 30-60 g/100 kg seed for cotton[84].

1.4.16 Prochloraz

Prochloraz (Molecular formula $C_{15}H_{16}Cl_3N_3O_2$, Molecular weight 376.7, CAS number 67747-09-5, melting point 48.0°C) is a member of the class of ureas that is 1H-imidazole-1-carboxamide substituted by a propyl and a 2-(2,4,6-trichlorophenoxy)ethyl group at the amino nitrogen atom. A fungicide active against a broad range of diseases affecting field crops, fruit, turf and vegetables. It has a role as a xenobiotic, an environmental contaminant, an EC 1.14.13.70 (sterol 14alpha-demethylase) inhibitor and an antifungal agrochemical. It is an aromatic ether, a trichlorobenzene, a member of ureas, a member of imidazoles, an amide fungicide, a conazole fungicide and an imidazole fungicide. Prochloraz is an imidazole fungicide that is broadly used in Europe, Asia, Australia, and South America within gardening and agriculture. Screening research have shown that prochloraz elicits multiple mechanisms of action in vitro, as it antagonizes the androgen and the estrogen receptor, agonizes the Ah receptor, and inhibits aromatase activity. Prochloraz acts as an antiandrogen in vivo (rat Hershberger assay) by reducing weights of reproductive organs, affecting androgen-regulated gene expressions in the prostate, and increasing LH levels.

1.5 Essential Oils

1.5.1 Essential oils Defined

Essential oils are aromatic, volatile liquids derived from plant material through steam distillation and named after the plant from which they are obtained. Essential oils can be defined as either products or mixtures of fragrant substances or as mixtures of fragrant and odorless substances. These fragrant substances are chemically pure compounds that are volatile under favorable conditions. Essential oils differ greatly, sometimes due to genetic causes, but also because of climate, rainfall, or geographic origin. They are composed principally of lipophilic and highly volatile secondary plant metabolites, principally mono- and sesquiterpenes, but other types of compounds such as allyl and isoallyl phenols may also be available. Other substances that have been identified in volatile oils include coumarins, anthraquinones, and alkaloids, which are often distillable, while some diterpenes, fat, and other nonvolatile compounds can be derived from essential oils by methods other than distillation. The applications of essential oils are diverse. Broadly used in cosmetics and perfumes, they also have medicinal applications due to their therapeutic properties as well as agro-alimentary uses because of their antimicrobial and antioxidant effects[85].

Since the mid-1980s significant attention has been given to essential oils for their potential use in alternative pest management of stored products. Existing synthetic pesticides including methyl bromide and phosphine which are used as fumigants for pest management of stored products have serious adverse properties. These include their toxicity against nontarget organisms, long half-life in the field, and contamination of air, soil, water, etc. In addition to these adverse effects, stored product pests develop resistance to these control agents. The growing world population and increasing food demand together

with increasing environmental concerns of society dictates an alternative method for the protection of precious food material. Essential oils produced by plants contain a vast array of secondary metabolites such as derivatives of monoterpenes, sesquiterpenes, diterpenes, aromatic compounds, hydrocarbons, and fatty acids. Plants yields essential oils for various purposes. Essential oils are used for attracting pollinators, deterring herbivores, effecting the growth of competitor plant species, and fighting fungal, bacterial, and viral plant diseases in plants. The volatile nature of essential oils and their known action on herbivores makes them a strong alternative in pest management of stored products[86].

1.5.2 Extraction Techniques

Essential oils are composite mixtures of volatile compounds most frequently found at low concentrations in plants. Several different extraction techniques are widely employed for the extraction of essential oils such as steam distillation and solvent extraction. These methods are characterized by drawbacks such as low extraction efficiency and selectivity, use of large amounts of solvents, and long extraction times. In many cases, the quality of the essential oil derived by conventional methods can be influenced by hydrolyzation or oxidation then can take place due to long extraction time and/or high water quantity. Due to these limitations, alternative methods for the extraction of essential oils have been developed which can typically overcome these problems. Supercritical fluid extraction, microwave assisted extraction and ultrasound are novel methods that are now recognized as efficient extraction methods and can significantly reduce extraction times, enhance yields, and quality of essential oil. Although these methods are predominantly exploited on the laboratory scale, they have also found industrial applications, although in most cases to a limited extent[87].

1.5.3 Essential oils as Fungicides

Some researchers have defined essential oils (EOs) as odorous products, generally of complex composition, obtained from a botanically defined plant staple, either by entrainment, by steam, or by dry distillation, or by an appropriate mechanical process without heating. It is approximated 30,000 plants can produce *essential oils* and over 150 differing kinds of oils are on the market. Known for many years, they're applied in many fields, like the food industry, perfumery, cleaning products, traditional medicine and aromatherapy. There has been a significant rise in interest in the research of essential oils due to their natural properties and consumer demand for safer and secure ways to preserve food. There are several essential oils varying in properties and application especially their antifungal and antimicrobial effects, however in this research, only some among the several kinds of essential oils were used. Essential oils used in this study included oranges, citronella, garlic, stone calamus, sweet orange, mugwort leaf, sandalwood, *Artemisia* annua, cedar. Some essential oils are noted for their floral scent and woody undertones. Properties include antibacterial, antidepressant, anti-inflammatory, antifungal, antiviral, antispasmodic.

1.5.4 Antifungal Action of Essential oils

The antifungal agents can make the fungus inactive by disordering the structure and performance of membranes or organelles of fungal cell or inhibiting the nuclear material or protein synthesis. The fungal cell membrane plays an important role within the growth and viability of fungi, the three major structural elements, glucan, chitin, and mannan, are overall considered therapeutic targets. Chitin, an extended linear homopolymer of β-1,4-linked N-acetylglucosamine (GlcNAc), is synthesized during a reaction catalysed by chitin synthase. Chitin is indispensable for the development of the plasma membrane, and consequently for fungal survival. The inhibition of chitin polymerization may affect cell membrane maturation, septum formation, and bud ring formation, by destroying organic process and cell growth.

Essential oils are characterized as secondary metabolites and usually fall under the category of *terpenes, ketones, esters, aromatic phenols, alcohols* and *oxides*. Essential oils act by inhibiting fungal hyphae growth by accumulating in the fungal cell membrane or by crossing the cell membrane and entering into the eukaryotic cell. Since the chemical natures of essential oils are lipophilic in nature they can easily cross the cell membrane and interrupt sterol biosynthesis causing growth retardation and finally

the death of the cell.

1.6 Research Trend

As an important and reliable food source, sweet potato can be used for commercial production, but also can be planted or grown by self-sufficient and small-scale farmers. The main producing areas of sweet potato in the world are distributed in the south of 40 degrees north latitude. The largest cultivated area is Asia, followed by Africa, and then America ranks third. Similar to other crops, sweet potatoes are susceptible to viruses, which are associated with large produce and quality losses. Soft rot and dry rot of sweet potato are the main diseases of sweet potato during storage. If not controlled, the disease will cause great harm to sweet potato, and finally lead to the quality loss and produce of sweet potato greatly reduced.

Many tests have been conducted in Louisiana and North Carolina to assess the effectiveness of the new chemicals commonly considered safe for the control of *Rhizopus stolonifer* soft rot, classified as (liver) or risk reducing fungicides or biological control agents[88]. Generally, liver products do not provide satisfactory control, while control biological control agents give some control but are lower than risk reduction fungicides, which is equivalent to botran providing control. A risk reducing fungicide was approved by the U.S. Food and Drug Administration for sweet potato in November 2008. However, in September 2008, the European Union and other countries set the maximum residue level of the chemical as the detection limit, which basically made it impossible for the product to be used in sweet potatoes in those markets. For disease to occur, all the components of the disease triangle must be available at the same time: susceptible host (in this case, sweet potato storage roots), pathogen (*R. stolonifer*) and favorable environment for disease occurrence. To manage it without relying on fungicides, a comprehensive project for each branch of the disease triangle is needed.

In the 1990s, researchers from LSU agricultural center began to screen several varieties of sweet potato for resistance to soft rot. These studies showed that Beauregard was more resistant than the old varieties. In a survey of commercial packaging lines, Beauregard could be packaged without fungicides in most cases, but in some cases, Rhizopus soft rot exceeded the 2% tolerance threshold set for the disease. For several years, efforts have been made to produce more resistant lines than Beauregard. Now, several lines have reasonable yield and stronger resistance. This will help to assess whether drug resistance can be used as a primary means of disease management. Resistance is the simplest and most economical tool for disease control. However, in the case of sweet potato soft rot, the expression of resistance will also be affected by several factors[89]. From 2003 to 2008, the U.S. Department of agriculture's risk aversion and mitigation program funded a large research involving scientists from the Louisiana State University Agricultural Center, Mississippi State University, North Carolina State University and Auburn University.

Sweet potato (*Ipomoea batatas* (L.) Lam) is grown throughout the tropics and subtropics, and ranks sixth or seventh among the most important food crops worldwide. Tabular Descriptions of Crops Grown in the Tropics. China produces about 80% of the yearly global output, where sweet potatoes ranks fourth as a food crop, after rice, wheat and maize. The trend in utilization of sweet potato in China is shifting away from its use as a staple food to use it as a processed food, a raw material for industrial products, and for feed products. In rural areas, the limited transport infrastructure has encouraged small-scale local processing and the development of feed uses of sweet potato which are primarily household-based. Although sweet potato is typically harvested in November or December, it is available for several months from storage in some areas in China[90].

Storing of sweet potato induces many changes in the carbohydrate fraction of the roots. The carbohydrate composition in sweet potato roots greatly affects the eating quality and processing properties in general, longer storage periods of raw roots prior to processing results in products with decreased firmness. Research, on the amylase activity in fresh and stored roots have been reported, a marked difference in individual and total sugar concentrations among sweet potato lines, and significant

varietal differences in α- and β-amylase activity during storage. Dreher reported that cereal starch is most susceptible to α-amylase digestion, while potato starch is resistant, and sweet potato starch has intermediate susceptibility. Significant variation in starch digestibility was seen among sweet potato genotypes. Thus, selection for genotypes with higher starch digestibility may be an effective way to increase sweet potato feed efficiency. Anti-nutritional factors such as trypsin inhibitors should also be taken into consideration. Trypsin inhibitors, proteinase inhibitors, which make proteins unavailable, are generally present in sweet potato roots. Variation in trypsin inhibitor activity among genotypes/cultivars has been reported[91].

Prolonged storage of unprocessed sweet potato roots is essential for food and feed availability. No comprehensive research have been carried out on the effects of storage time on digestibility, starch pasting properties, and trypsin inhibitor activity of diverse genotypes. In August 2011, sweet potato (*Ipomoea batatas*), tomato (*Solanum lycopersicum*), and eggplant (*S. melongena*) crops from major growing areas of the Cameron highlands and Johor state in Malaysia were affected by a soft rot disease. Disease incidence exceeded 65, 75, and 80% in severely infected fields and greenhouses of sweet potato, tomato, and eggplant, respectively. The disease was characterized by dark and small water-soaked lesions or soft rot symptoms on sweet potato tubers, tomato stems, and eggplant fruits. In addition, extensive discoloration of vascular tissues, stem hollowness, and water-soaked, soft, dark green lesions that turned brown with age were found on the stem of tomato and eggplant. A survey was conducted in these growing areas and 22 isolates of the pathogen were derived from sweet potato (12 isolates), tomato (6 isolates), and eggplant (4 isolates) on nutrient agar (NA) and eosin methylene blue (EMB). The cultures were incubated at 27°C for 2 days and colonies that were emerald green on EMB or white to gray on NA were selected for further research.

Sweet potato also known as *Ipomoea batatas* Lam, is an annual or perennial vine. Root tubers can be used for several functions such as food, feed and industrial raw materials. As an important and reliable food source, sweet potato can also be used for commercial production, but also can be grown by self-sufficient and small-scale farmers. The main producing areas of sweet potato in the world are distributed in the south of 40 degrees north latitude and the largest cultivated area is Asia, followed by Africa, and then America takes the third position. In 1985, the cultivating area of sweet potato was 80.3 million hectares, and the total production was 111.438 million tons. Sweet potato is broadly distributed in China, especially in Huaihai plain, Yangtze River Basin and southeast coastal provinces as described in the introduction above.

Some renowned researchers like J.B. Edmund and others not to mention many believed that sweet potato is an origin crop of Mexico and tropical America from Colombia, Ecuador to Peru. A.Von Humboldt quotes From Gomarra: When Columbus first paid a visit to the Queen of Spain, he presented her with sweet potatoes brought back from the New World. At the very beginning of the 16th century, sweet potatoes were widely planted in Spain. Spanish sailors carried it to Manila and Molucca in the Philippines, where it widely spread throughout Asia. Sweet potato was introduced into China via several channels, and it was around the end of the 16th century. There are relevant records in Min Shu of the Ming Dynasty, Fu Shu of Rural Administration of The Ming Dynasty, Fu Shu of Min Zheng of the Qing Dynasty and Fu Zhi of Fuzhou. Chen Shiyuan of the Qing Dynasty cited the Record of The Gathering of Min Hou He zhi in his Biography of Golden Potato: "It was cultivated overseas during the Reign of Wanli in the Ming Dynasty, Chen Zhenlong, a min man, traded his land, got the vine seedlings and planted them into China. Mid - Fujian drought hunger. Longzi Zhen and Jinglun Bai, when the governor Jin Xuecheng ordered him to try to plant it, produced a great deal, which could be filled with half of the grain. The sterile field has been proof against seeding everywhere." He also said, "I got it from the Old Chinese name yams. It is planted with gold, so it is also called golden potato." According to further information, Chen Shiyuan, the 6th grandson of Chen Zhenlong, and his son Chen Yun were successively cultivated with sweet potato in Yinzhou (Ningbo, Zhejiang), Jiaozhou, Qingzhou (Qingdao, Yidu, Shandong province) and Yuzhou

(Zhuxian Town, Henan Province), and gradually spread throughout Zhejiang.

The above historical facts proves that sweet potato was introduced from Nanyang to Fujian and Guangdong in the late 16th century, and then widely spread to the Yangtze River, Yellow River basin and Taiwan province according to the research findings. China has the world's largest acreage and total output of sweet potatoes. The main producing areas of sweet potato in the world are distributed south of 40° N. Asia had the largest planted area, then followed by Africa, and America came in third. In 1985, the planted area of sweet potato was 800.3 million hectares, and the total output was 111.438 million tons. Sweet potato is broadly distributed in China, with huaihai Plain, Yangtze River basin and southeast coastal provinces most. The whole country is divided into 5 potato areas: Northern spring potato areas. Including Liaoning, Jilin, Hebei, Northern Shaanxi and other places, the region is short frost-free period, low temperature approach early, cultivated more spring potato. The potato region in spring and summer of Huanghuai Basin. It belongs to the warm temperate climate of monsoon. It is more favorable to grow potatoes in spring and summer. The planting area accounts for about 40% of the total area of the country. Summer potato area in the Yangtze River Basin. The whole Yangtze River basin except qinghai and northwest Sichuan plateau. Southern summer and autumn potato area. North of the Tropic of Cancer, south of the Yangtze River basin, in addition to planting summer potato, some areas also grow autumn potato. Southern autumn and winter potato region. The coastal land to the south of the Tropic of Capricornus and The islands such as Taiwan belong to the humid tropical climate, high temperature in summer and small temperature difference between day and night, mainly planting autumn and winter potato. The cropping system differs from region to region in China.

Sweet potato soft rot disease or Rhizopus soft rot is caused by the necrotrophic, Zygomycete fungus *Rhizopus stolonifer*. *R. stolonifer* is a ubiquitous fungus that causes postharvest soft rot on more than 200 fruit and vegetable crops, mostly observed in sweet potato and stone-fruits. Rhizopus soft rot typically appears during postharvest handling, transporting and is rarely seen in the field. Symptoms usually originate at a wounded area in the sweet potato and consist of a soft, watery rot that progresses quickly under suitable conditions and can result in full decay of an infected root in as little as three days. White to grey fungal mycelium producing black sporangia of *R. stolonifer* are often observed growing on decayed roots, resembling a distinctive "grey man's beard." *R. stolonifer* requires wounds in the sweet potato root, particularly bruising wounds, for the disease to initiate. Disease progression and sporulation are then significantly influenced by storage conditions[92]. While disease can occur over a wide range of temperatures, elevated temperatures of 25-29°C suit for disease progression. Storage of sweet potatoes at relative humidity between 75-100% seems to have little or no effect on disease progression, however higher humidity significantly increase sporulation of *R. stolonifer*, leading to increased secondary inoculum{Sallato, 2007 #109}. Likewise, initial inoculum levels also have little impact on disease progression but do promote higher amounts of secondary inoculum. *R. stolonifer* is a ubiquitous fungus, thus difficult to eliminate. Considering this is primarily a postharvest disease, it is often transmitted due to unsanitary harvest equipment, wash lines, packing equipment, and transportation containers.

According to some research, prevention is the best way for control of Rhizopus soft rot and several steps can be taken to eliminate disease outbreaks such as; reduce wounding during harvest, washing, and packing. Cure roots in a timely manner after harvest to allow for proper wound healing. Store sweet potatoes at 85% humidity and 13°C with proper ventilation. Maintain sanitary harvesting, storage, washing, and packing equipment. Growers with established protocols to daily clean facilities and tools experience few challenges with Rhizopus soft rot. Eliminate infected roots from entering wash tanks and packing lines. Quickly remove discard bins or culls away from healthy roots in a way such that infected roots with sporulation are not expose.

1.7 Aim and Significance

Sweet potatoes are widely cultivated in many countries around the world and have the 7th largest production of the world's total food production. Sweet potato soft rot is one of the main diseases during the storage period of sweet potato, which has a serious impact on the yield and quality of sweet potato, and causes huge losses to the production of food crops in China. At present, the main way to control sweet potato soft rot is still the application of chemical fungicides, and it is not clear which fungicide has the best control effect among the common fungicides. In this study, the agents with the best effect on the prevention and control of soft rot in sweet potatoes were screened through indoor antibacterial activity test. However, fungi are very resistant to chemical fungicides, and considering that chemical fungicides can have adverse effects on the ecological environment and food safety. Therefore, the development of green biopesticides is an inevitable trend to control plant diseases, because plant essential oils have the characteristics of naturalness, safety and efficiency, have been widely used in a variety of fruits and vegetables postharvest disease control, and show good sterilization effect and preservation effect. At present, the antibacterial activity of essential oils on Rhizopus stolonifer is not well studied. Therefore, this paper tests the bacteriostatic activity of fungicides and plant essential oils with good antibacterial effect on Rhizopus stolonifer, and tests the indoor control effect by compounding them, explores the best scheme of fungicide and essential oil compounding, and provides a combination of chemical and biological control in the prevention and control of sweet potato soft rot, which not only has good control effect, but also benefits the ecological environment. It provides a theoretical basis for the compounding of chemical fungicides and plant essential oils in the prevention and control of sweet potato soft rot, and also provides a theoretical basis for the future development.

2 Identification of the fungus by observing the Sporeand Hyphal

2.1 Materials and Methodology

2.1.1 Materials

Different materials were used for the identification of strains which included potato dextrose agar (PDA), petri dish, soft rot fungus and YG10 Olympus microscope etc.

2.1.1.1 Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) was used for cultivation of fungal strains. Potato Dextrose Agar is composed of dehydrated potato infusion and dextrose that encourage luxuriant fungal growth. Agar was added as the solidifying agent. Potato dextrose agar media were chosen for studying morphological characteristics.

2.1.1.2 Soft Rot Fungus

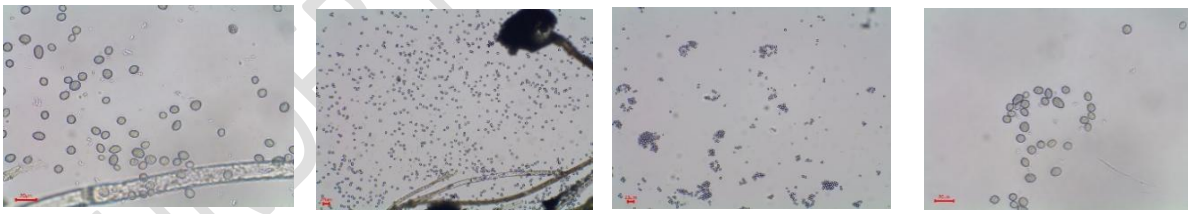
Samples of soft rot fungus were cultured on PDA. Soft rot is caused by a necrotrophic, Zygomycete fungus *Rhizopus stolonifer*. Rhizopus soft rot typically appears during postharvest handling and transport and is rarely observed in the field. Symptoms originate at the wounded area of sweet potatoes and consist of a soft, watery rot.

2.1.2 Method

PDA was used for cultivation of fungi and studying morphological characteristics. The plates were kept at 26°C in an incubator and morphological properties of fungi and were observed. After 2-3 days the fungi on the plates matured, the fungal strains were observed on a microscope. The microphotograph of fungus was taken by YG10 microscope software inbuilt in binocular compound microscope.

2.2 Results

The Figures 5.1 and 5.2 below shows the results of the identification of the strains by observing the spore morphology and hyphal morphology. After 2-3 days the fungi on the plates matured, the fungal strains were observed under a YG10 microscope. The microphotograph of fungus was taken by YG10 microscope software inbuilt in binocular compound microscope.



A B C D

Figure 4.1 Spores

Spore figure A is more magnified by x40 than spore figure B and spore figure D is more magnified by x40 than spore figure C.

The Figure 4.1 above shows the picture of the spores taken using a microscope, spore figure A is more magnified by x40 than spore figure B Spore figure C and spore figure D were taken on the other part of which spore figure D is more magnified by x40 than spore figure C The spores belonging to rhizopus were identified and seen on the microscope.

A B C D

Figure 4.2 Hyphae

The above Figure 4 shows the hyphae taken using a microscope, the 4 pictures shows different parts of the hyphae. The hyphae belonging to rhizopus was identified and seen on a microscope.

2.3 Summary

In this section, the main aim was to identify the species by observing the spore morphology and hyphal morphology. After the fungi on the plates matured, the fungal strains were observed on a microscope. The microphotograph of fungus was taken by YG10 microscope software inbuilt in binocular compound microscope. The spores belonging to rhizopus were identified. In Figure 4.2 the 4 pictures shows different parts of the hyphae. The hyphae belonging to rhizopus was identified and seen on a microscope.

3 Highly effective green prevention and control methods of sweet potato soft rot disease by screening different kinds of control agents

3.1 Materials and Methodology

3.1.1 Sample Collection

Materials used like potato tubers and biocontrol agents were obtained from Modern Agricultural College of Zhejiang Agricultural and Forestry University.

3.1.2 Materials

Potato dextrose agar (PDA) was used to cultivate the sweet potato soft rot. Control agents used in the study included Tebuconazole, Procymidone, Pyraclostrobin, Prochloraz, Kresoxim-methyl, Azoxystrobin, Prothionazole, Octreotide, Boscalid, Difenoconazole, Thifluzamide, Carbendazim, Pyraclostrobin and Triadimenol.

3.1.3 Methods

The growth rate method was used to determine the inhibitory effect of different agents on sweet potato soft rot. Each fungicide or control agent (Tebuconazole, Procymidone, Pyraclostrobin , Prochloraz, Kresoxim-methyl, Azoxystrobin, Prothionazole, Octreotide, Boscalid, Difenoconazole, Thifluzamide, Carbendazim, Pyraclostrobin and Triadimenol) was used at different concentration of 1 μg/mL, 1.25 μg/mL, 2 μg/mL, 5 μg/mL and at 20 μg/mL. Firstly, tools and PDA solution were sterilized. In the sterile workbench, each agent was diluted with sterile water to the required concentration. After shaking well, 1mL diluent was taken into a Petri dish, then 9mL PDA solution was added to make the drug containing medium, in the meanwhile 1mL sterile water was added to 9mL PDA solution serving as the control group. After the medium was solidified, the punch was used to make and transfer the fungal plugs, the mycelium plugs was put face down, inoculated in the center of each treated medium. Each treatment was repeated for 3 times, and plates were placed in a 25 ℃ constant temperature incubator in the dark. On the third day, the diameter of the mycelium colony was measured for each treatment containing different agents as well as control. The growth inhibition rate of each agent treatment on colony expansion was calculated, and the effects of different agents on the growth of sweet potato soft rot pathogen were analyzed and compared. The growth inhibition rate of the treatment of each agent on colony expansion is calculated by comparing with the control, and the effects of different agents on the growth of sweet potato soft rot fungus are analyzed and compared.

Inhibitory rate (%) = [(control colony diameter -0.5)-(treatment colony diameter -0.5)]/ (control colony diameter -0.5) ×100

3.2 Results

3.2.1 Average Diameter at 20 μg/mL, 5 μg/mL, 2.5 μg/mL, 1.25 μg/mL and 1 μg/mL Concentration

The results showed (see Figure 6.1) that at a concentration of 20 μg/mL, the inhibitory rate of Triadimenol (三唑醇), Pyraclostrobin (唑菌酯), Kresoxim-methyl (醚菌酯), Prothionazole (丙硫菌酯), Carbendazim (多菌灵), Azoxystrobin (嘧菌酯), Octreotide (辛菌胺) inhibition rate is less than 50%, Carbendazim 多菌灵) as low as 1.2% and Octreotide (辛菌胺 had no inhibitory activity for sweet potato soft rot. The other 7 agents have good inhibitory activity for sweet potato soft rot.

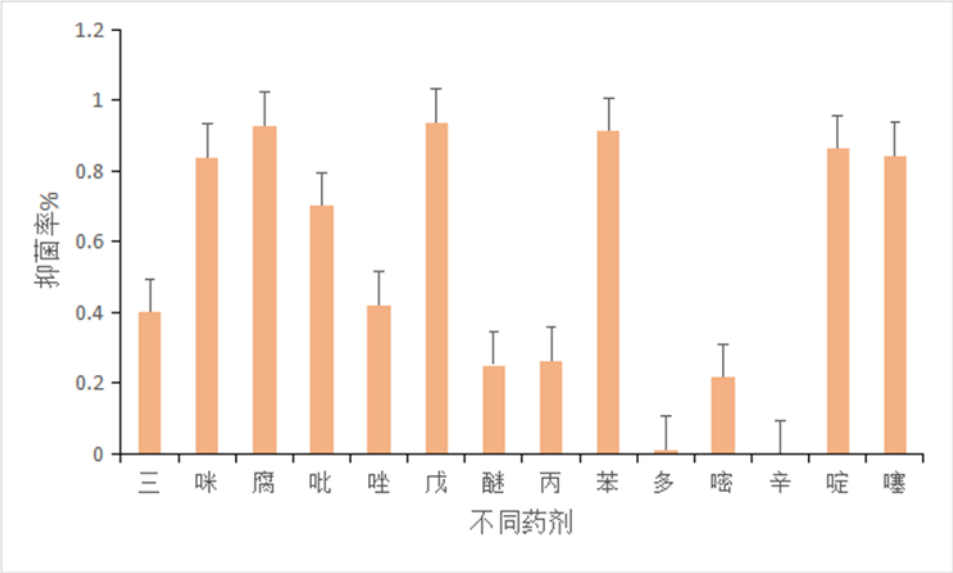


Figure 5.1 Inhibitory rate of sweet potato soft rot at a concentration of 20 μg/mL by different agents

At a concentration of 5 μg/mL, Pyrazoxytrobin (吡唑醚菌酯), Tebuconazole (戊唑醇), Difenoconazole (苯醚甲环唑), Boscalid (啶酰菌胺), Thifluzamide (噻呋酰胺) showed good control effects on sweet potato soft rot, with a prevention effect of 71.8%, 88.6%, 82.0%, 65.9% and 94.9%, respectively. The result is shown in Figure 5.2

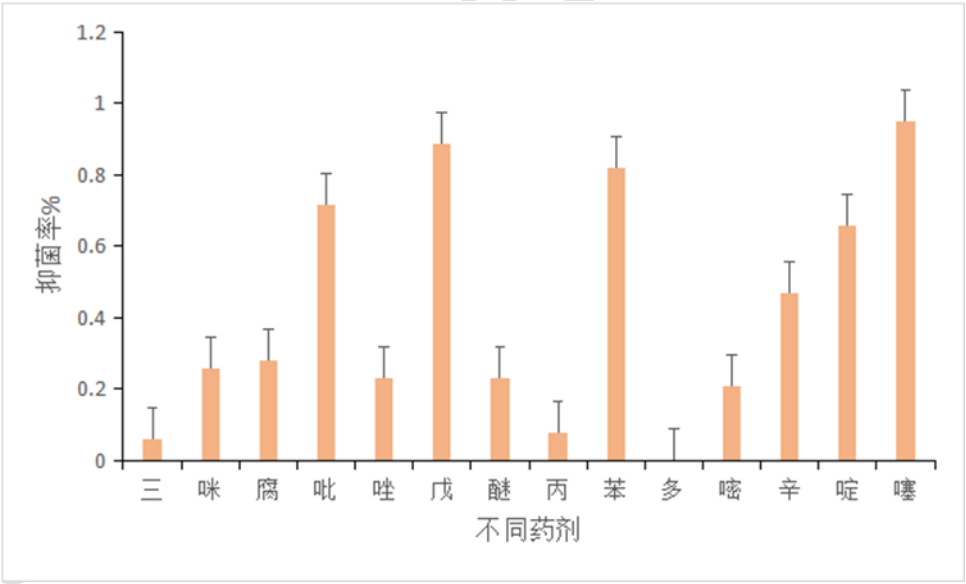


Figure 5.2 Inhibitory rate of sweet potato soft rot at a concentration of 5 μg/mL by different agents

At a concentration of 2.5 μg/mL, the above-mentioned fungicides and broad-spectrum fungicides fluzinam (腐霉利) and fludioxonil (咯菌腈) were selected to carry out toxic plate experiments with sweet potato soft rot as an indicator fungus, and the Inhibitory rates of thifluzamide (噻呋酰胺), fludioxonil (咯菌腈), difenoconazole (苯醚甲环唑), pyrazoxytrobin (吡唑醚菌酯), boscalid (啶酰菌胺), and tebuconazole (戊唑醇), were 31.8%, 89.8%, 52.9%, 61.2%, 20.4%, 22.7%, 80.8%, respectively, from the above, it can be seen that thifluzamide, pyrazoxytrobin, boscalid, the Inhibitory

effect at 20 μg/mL and 5 μg/mL concentrations is very good, but as the concentration decreases, the inhibitory activity also decreases. The difenoconazole and tebuconazole still maintain good Inhibitory activity. In addition, the broad-spectrum fungicide fluazinam also has a good Inhibitory effect on sweet potato soft rot. This is shown in Figure 5.3

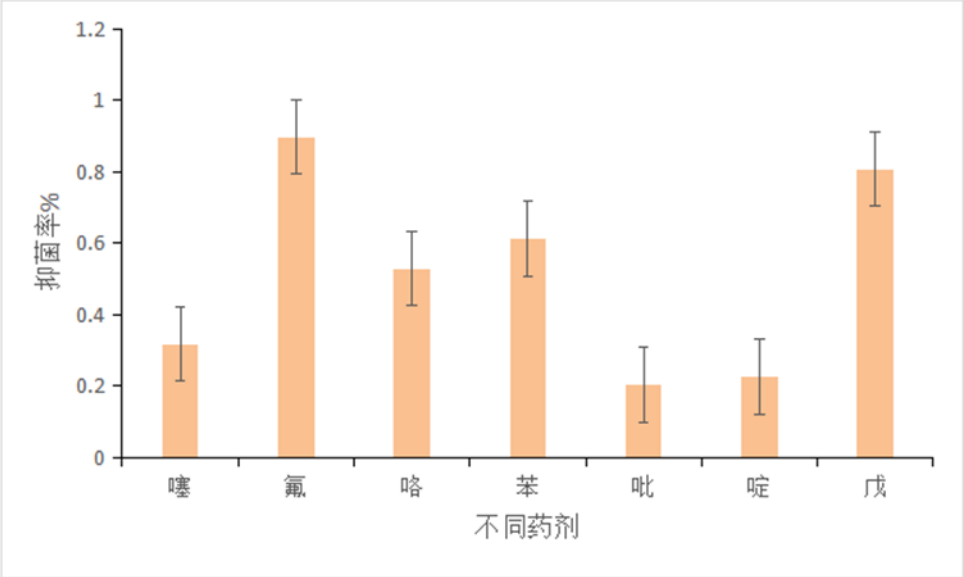


Figure 5.3 Inhibitory rate of sweet potato soft rot at a concentration of 2.5 μg/mL by different agents

When the concentration was reduced to 1.25 μg/mL, the Inhibitory activity of difenoconazole (苯醚甲环唑), also decreased, the Inhibitory rate reached 12.2%, the Inhibitory rate of tebuconazole(戊唑醇), was still maintained at 62.9%, and the Inhibitory activities of thifluzamide (噻呋酰胺), pyrazoxystrobin (吡唑嘧菌酯), and boscalid (啶酰菌胺), also decreased, 7.6%, 16.0%, and 23.6%, respectively. The result is shown in Figure 5.4

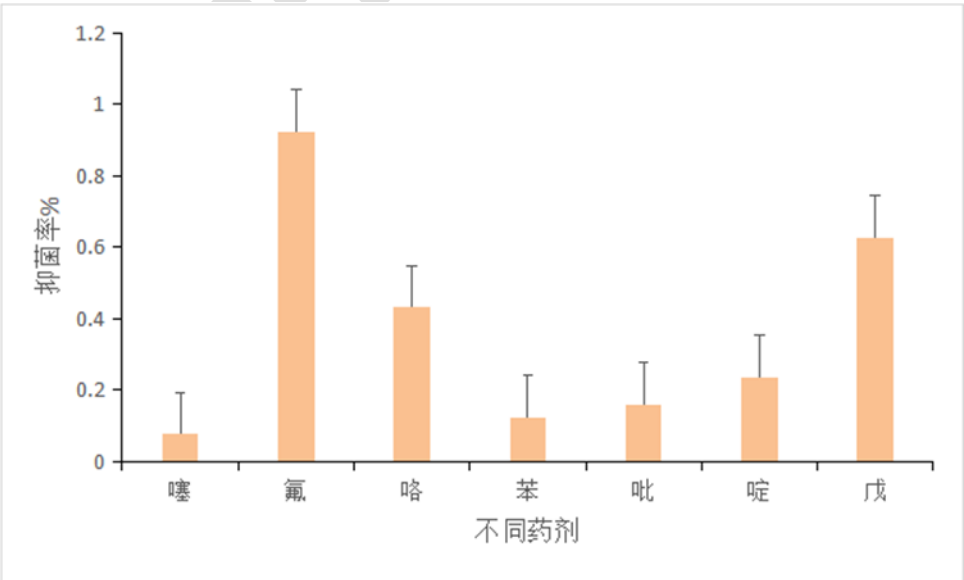


Figure 5.4 Inhibitory rate of sweet potato soft rot at a concentration of 1.25 μg/mL by different agents

When the concentration dropped to 1 μg/mL, fluazinam (腐霉利) and tebuconazole (戊唑醇), still maintained good Inhibitory activity, 92.4% and 62.9%, respectively, while when the concentration of the crystal eye decreased, the Inhibitory activity increased, reaching 43.1%. The Inhibitory activities of thifluzamide (噻呋酰胺), difenoconazole (苯醚甲环唑), pyrazoxytrobin (吡唑嘧菌酯), and boscalid (啶酰菌胺), were still very low, 8.6%, 4.3%, 20%, and 0.4%, respectively. The results are shown in Figure 5.5

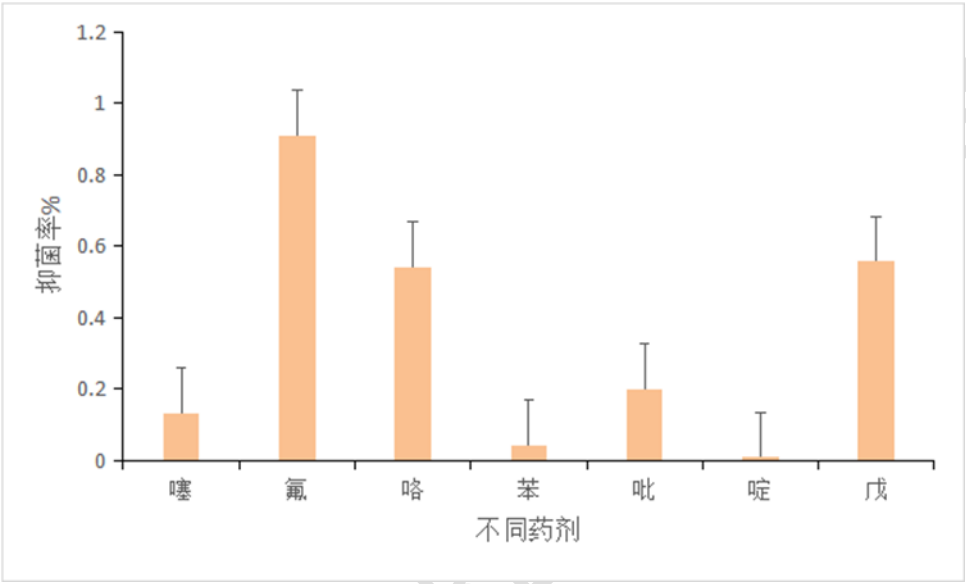


Figure 5.5 Inhibitory rate of sweet potato soft rot at a concentration of 1 μg/mL by different agents

The figure 6 below shows pictures of the results of the average diameter at different concentration taken using the canon digital camera.

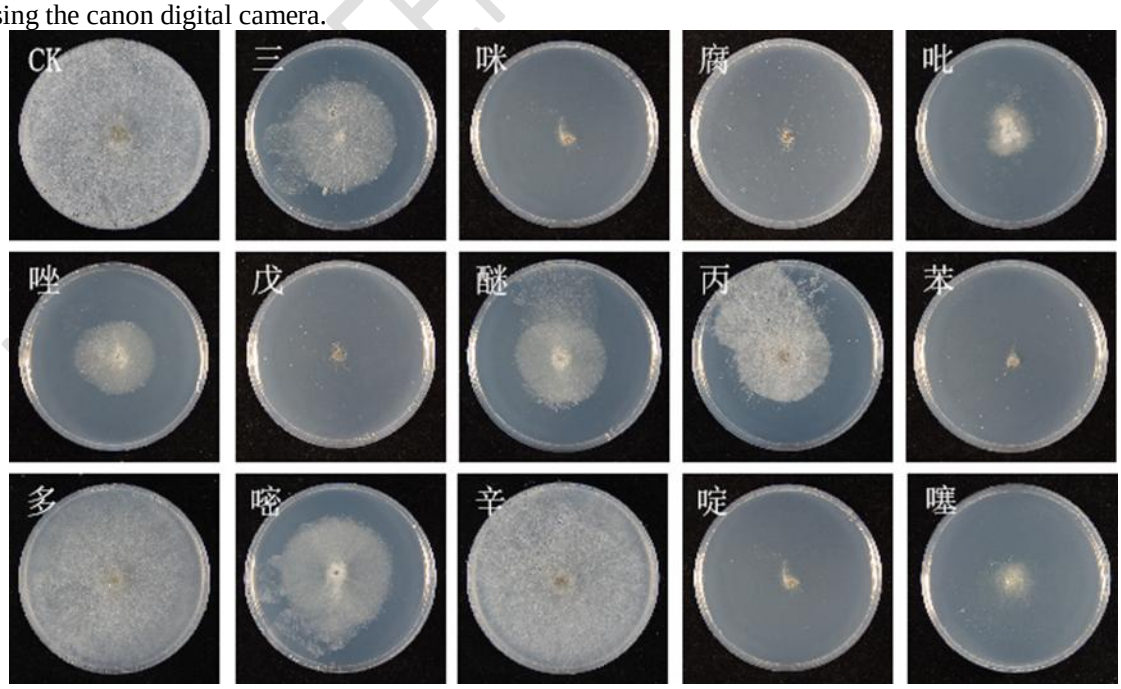


Figure 6.1 Initial screening for control of soft rot of sweet potato（ 20 μg/mL）

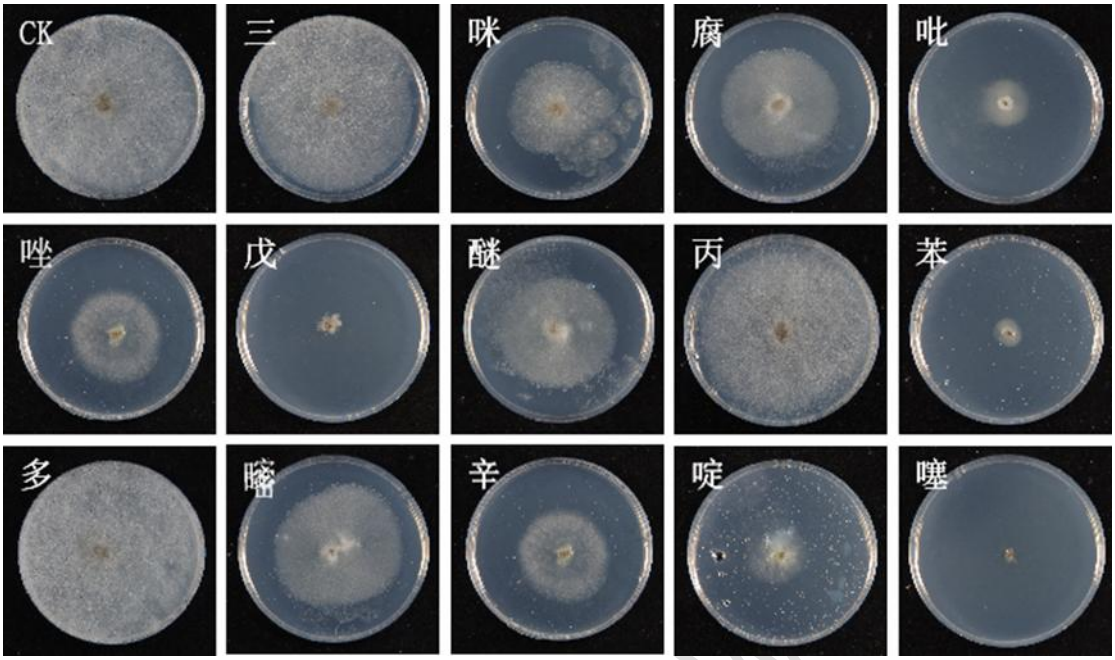


Figure 6.2 Initial screening for control of soft rot of sweet potato（ 5 μg/mL）

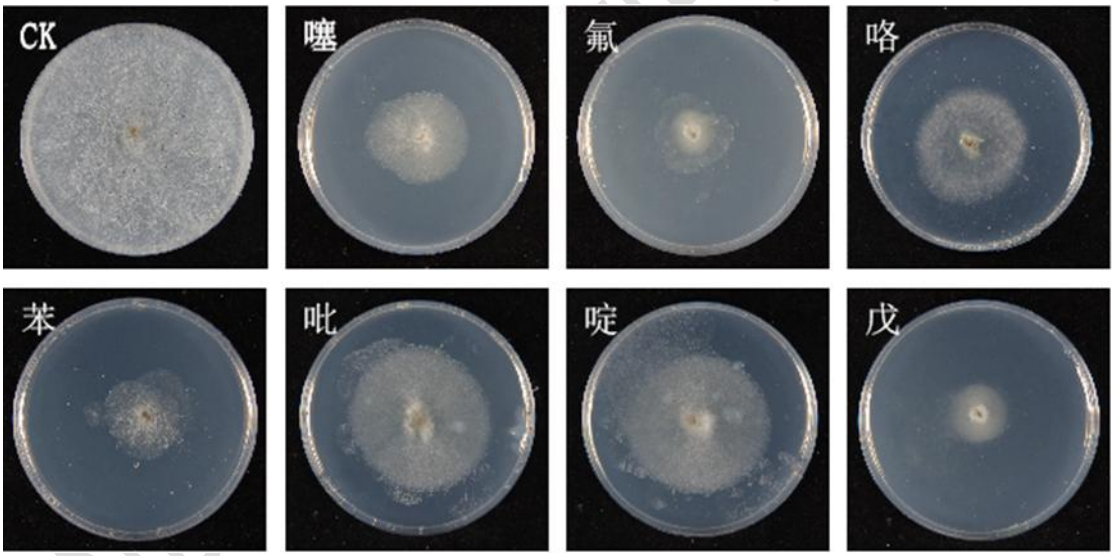


Figure 6.3 Initial screening for control of soft rot of sweet potato（ 2.5 μg/mL）

Figure 6 above shows pictures of morphology on each control agent at different concentrations, indicated by an average diameter of the colony. 3 pictures of each control agent were taken in total and one of each has been used to represent each control agent.

3.2.2 Data Analysis

Fluazinam, is an organic compound with the molecular formula $C_{13}H_4Cl_2F_6N_4O_4$ and a molecular weight of 465.092, pale yellow crystal. It is a pyridinamine derivative, dinitroaniline fungicide. With no therapeutic effect and aspiration activity, it is a broad-spectrum and highly effective protective fungicide. It is very effective against crospores, *Phytophthora*, *Unicytogenes*, and *Nigrass*. It also has a good effect on the gray spores of benzimidazole and dicarboxyimide fungicides, and also has a good effect on rice cataplexy caused by rhizobium. This product is extremely resistant to rain washing and has a long residual

effect period. It also has the effect of controlling plant-eating mites. Because β-trifluoromethylaminopyridine partially plays a unique role in transporting compounds to the active point of pathogenic fungi, it can inhibit the germination, penetration, growth of hyphae and spore formation during infection.

Tebuconazole is an organic compound with the molecular formula $C_{16}H_{22}ClN_3O$, which is a highly efficient, wide-spectrum, systemic triazole fungal pesticide with three functions of protection, treatment and eradication, with a broad fungal spectrum and a long effective period. Like all triazole fungicides, pentazole is able to inhibit the biosynthesis of ergosterols of fungi. Pentazole is used worldwide as a seed treatment agent and foliar spray, with a wide spectrum of sterilization, not only high activity, but also a long shelf life. Pentazole is mainly used to control a several variety of fungal diseases on crops such as wheat, rice, peanuts, vegetables, bananas, apples, pears and corn sorghum, and it has been registered and broadly used in more than 60 crops in more than 50 countries around the world.

In conclusion this section shows us the detailed results and data analyses of this research on high effective green prevention and control methods of sweet potato soft rot disease at different concentrations by screening different kinds of control agents or fungicides namely; Tebuconazole, fludioxonil, Procymidone, Fluazinam, Pyrazoxystrobin, Prochloraz, Kresoxim-methyl, Azoxystrobin, Prothionazole, Octreotide, Boscalid, Difenoconazole, Thifluzamide, Carbendazim, Pyraclostrobin and Triadimenol. The experiments were conducted in the laboratory and results suggested that two fungicides Fluorozilamine and Tebuconazole have good inhibition rate implying that they have higher prevention and control effect of sweet potato soft rot disease.

3.2.3 EC_{50} Value

The EC_{50} value is the concentration of a drug that produces half-maximal response. This section shows us the Ec_{50} value of the 3 types of fungicides Tebuconazole 戊唑醇, Fluazinam氟啶胺 and Fludioxonil咯菌腈. These values were calculated using SPSS application.

3.2.3.1 Tebuconazole 戊唑醇

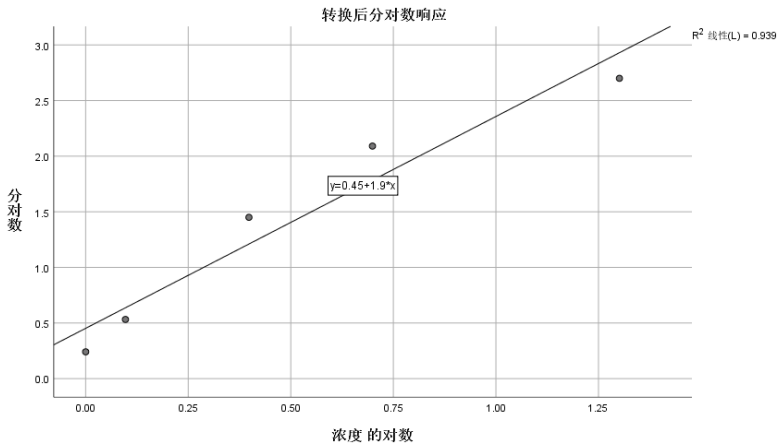


Figure 7.1 Tebuconazole EC_{50} graph

Above is the graph showing the EC_{50} value of Tebuconazole which was computed using the SPSS application. Its EC_{50} value was 692 μg/mL, toxicity regression equation is y=0.45+1.9*x and R^2 is 0.939.

3.2.3.2 Fluazinam氟啶胺

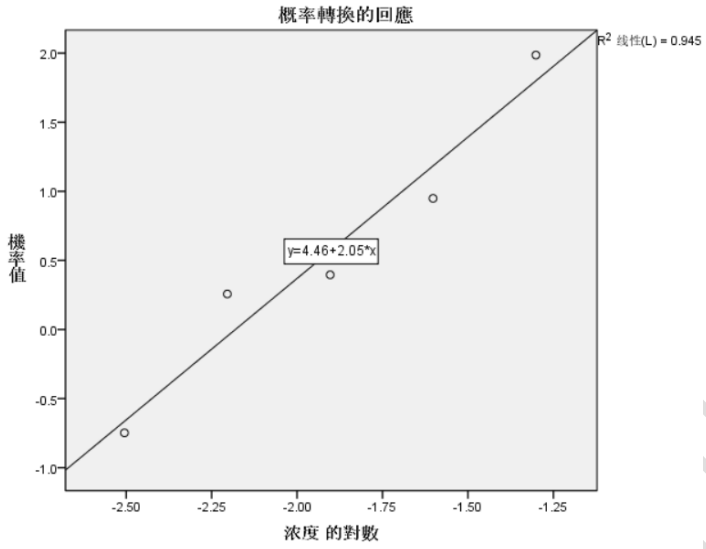


Figure 7.2 Fluazinam EC_{50} graph

Above is the graph showing the EC_{50} value of Fluazinam which was computed using the SPSS application. Its EC_{50} value was 6.418 μg/mL, toxicity regression equation is y=4.46+2.05*x and R^2 is 0.945.

3.2.3.3 Fludioxonil 咯菌腈

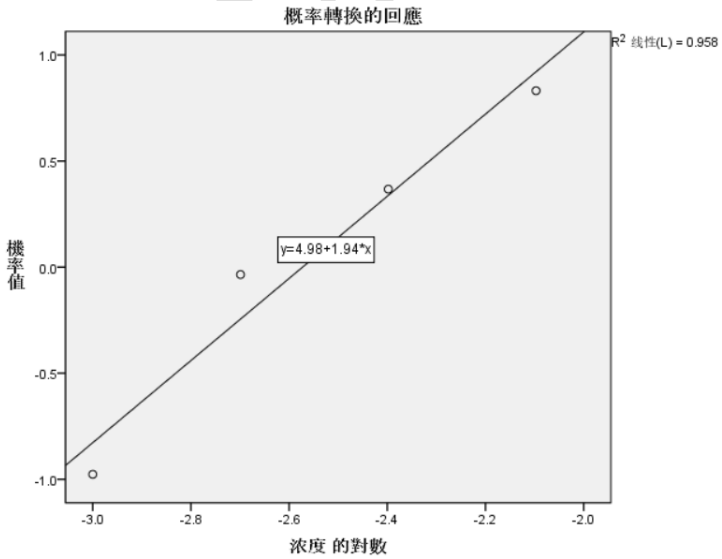


Figure 7.3 Fludioxonil EC_{50} graph

Above is the graph showing the EC_{50} value of Fludioxonil which was computed using the SPSS application. Its EC_{50} value was 2.509 μg/mL, toxicity regression equation is y=4.98+1.94*x and R^2 is 0.958.

3.2.3.4 Data Analysis

EC_{50} was computed for 3 types of fungicides Tebuconazole, Fluazinam and Fludioxonil , and were calculated using SPSS application. Results found shows Tebuconazole with toxicity regression equation of y=0.45+1.9*x and R^2 is 0.939, Fluazinam with toxicity regression equation of y=4.46+2.05*x and R^2 is 0.945 and Fludioxonil with toxicity regression equation of y=4.98+1.94*x and R^2 is 0.958.

4 Highly effective green prevention and control methods of sweet potato soft rot disease by screening different kinds of Essential Oils

4.1 Materials and Methodology

4.1.1 Materials

Potato dextrose ager (PDA) was used to cultivate sweet potato soft rot. Other materials used included burner, ultra clean table, alcohol lamp, punch, flask, small rectangular shaped papers. Essential oils used in this study included oranges, citronella, garlic, stone calamus, sweet orange, mugwort leaf, sandalwood, *Artemisia* annua, cedar.

4.1.2 Method

The 9 different essential oils mentioned above were tested by adding them to the soft rot culture on PDA. Specifically a droplet of each oils was added on the rectangular white paper stuck inside the lid of the fungal culture plate. Each oil was repeated 3 times. It took 6-7 days for the whole experiment to be successfully conducted and to be obtain the results. Average diameter of colony of each treatment was measured, and inhibition rate was calculated.

4.2 Results

Results of effects of 9 different essential oils on managing the sweet potato soft rot are shown in the table below. The experiment aimed at exploring the effects of these essential oils on the prevention and control of this fungus.

Table 2. Essential Oils and their average and inhibition rate

ESSENTIAL OILS	***AVERAGE DIAMETER(CM)***	***INHIBITION RATE***
ORANGES	7	0.91
CITRONELLA	9.67	0.88
GARLIC	17.67	0.79
STONE CALAMUS	5	0.94
SWEET ORANGE	76.67	0.08
MUGWORT LEAF	61	0.27
SANDALWOOD	71.33	0.14
ARTEMISIA ANNUA	73	0.12
CEDAR	78	0.06
CK	85	0

(Source: Authors own computation)

The table above shows the average diameter and inhibition rate for the essential oils. Stone calamus, oranges, citronella and garlic were found to have a higher inhibition rate implying that they have better inhibitory effect on the fungus.

4.3 Summary

The experiment on 9 different essential oils aimed at exploring the effects of these essential oils on the prevention and control of this fungus. Among the 9 different essential oils tested, stone calamus, oranges, citronella and garlic were found to have a higher inhibition rate implying that they have better inhibitory effect on the fung

5 Determination of indoor control effect of fungicides combined with plant essential oils on sweet potato soft rot disease

5.1 Materials and Methodology

Three white counting boxes were prepared, three layers of paper towels were spread on the bottom, then poured sterile water to moisten the paper towels and set aside. 100mg of large mountain sweet potatoes was selected, then washed and wiped off the surface water with a paper towel, left them at room temperature for 10 minutes, and then used a scalpel to cut 2 wounds of 2cm on the surface of each small sweet potato, inoculated the cultivated Rhizopus creeping sticky mushrooms with two 1m3 bacterial blocks on the wounds of the small sweet potatoes and then sprayed the following prepared pesticides on the surface of the small sweet potatoes, with three replicates for each treatment.

Fungicides+plant essential oils formulation

Control: Clean water treatment

Treatment 1: Fludioxonile 125 μL + Calamus Essential Oil 125 μL + Tween-80 2 mL + 26.125 mL

Treatment 2: Fludioxonile 250 μL + Calamus Essential Oil 250 μL + Tween-80 2 mL+ Water 6.25 mL

Dosage: 10 mLmixture

5.2 Results

The results showed that when the storage temperature of sweet potato was at the room temperature, the soft rot disease index was the lowest. When the sweet potato callus temperature was at the room temperature, and the potato cube wound started healing in 2-4 days thereby reducing the risk of pathogenic infection. During storage, spraying of Fludioxonile 125μL + Calamus Essential Oil 125 μL + Tween-80 2 mL+ 26.125 mL and Fludioxonile 250 μL + Calamus Essential Oil 250μL + Tween-80 2 mL + Water 6.25 mLhad good control effects on soft rot of sweet potato. When the storage time is 60 days, the control effect can still be achieved.

5.2.1 Sweet Potatoes before Inoculation

Figure 8 Sweet potatoes before Inoculation

The above figure shows mountain sweet potatoes before inoculation.

5.2.2 Sweet Potato Inoculated with Rhizopus Creeping for 2 days

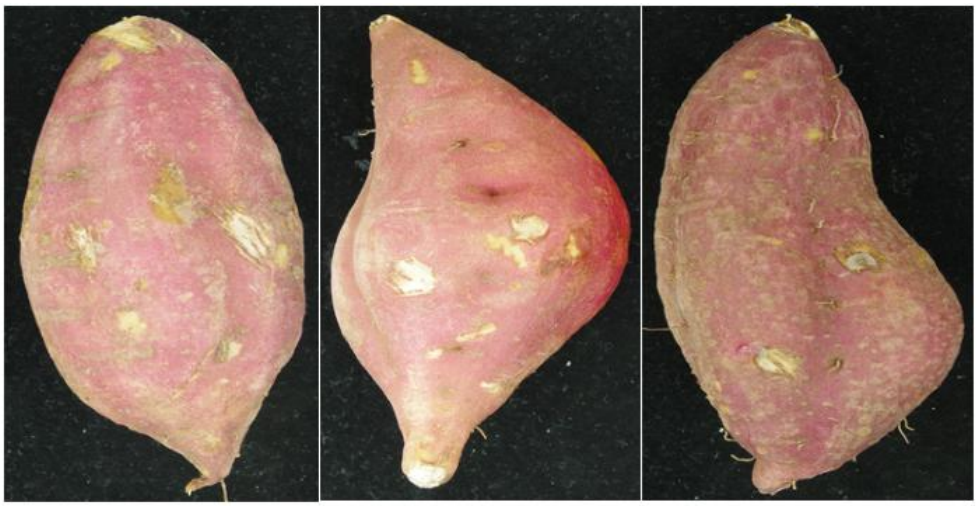


Figure 9.1 CK

Figure 9.2 Treatment 1

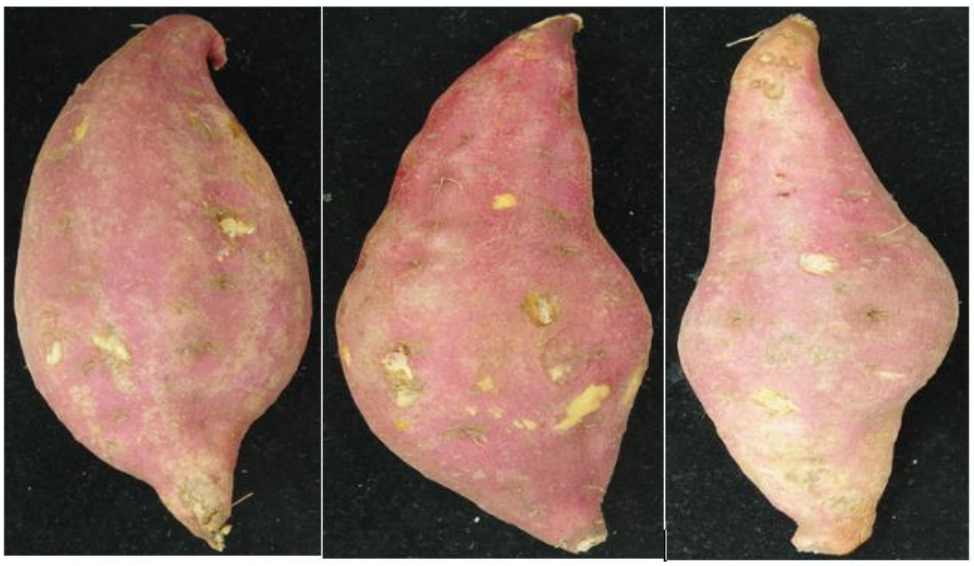


Figure 9.3 Treatment 2

The figures above CK, treatment 1 and treatment 2 shows the sweet potatoes inoculated with Rhizhopus creeping for 2 days. From the 2 days observation the fludioxonile pesticide plus calamus essential oil

treatment had a good control and preventive effect on sweet potatoes.

5.2.3 Sweet Potato Inoculated with Rhizopus Creeping for 10 days

Figure 10.1 CK

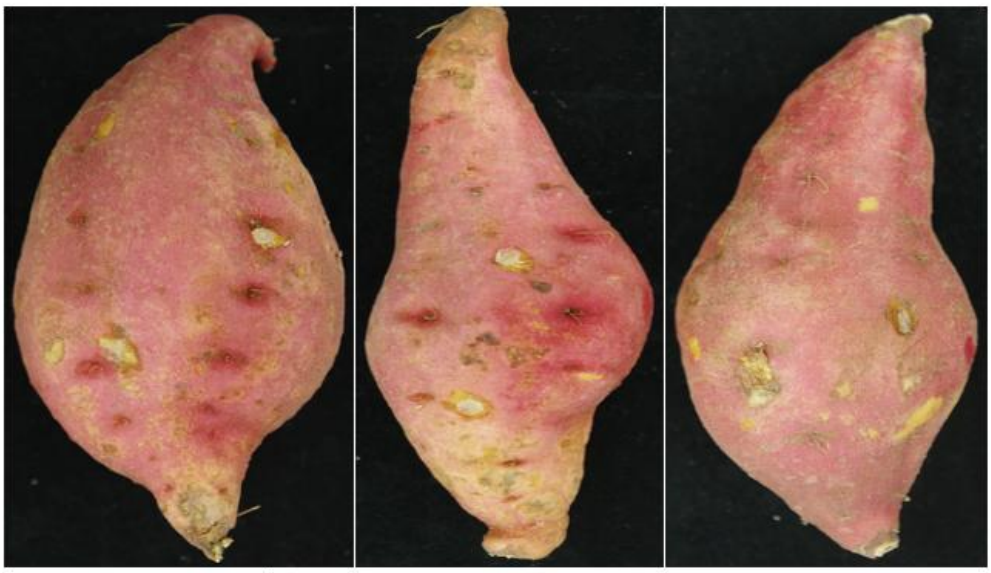


Figure 10.2 Treatment 1

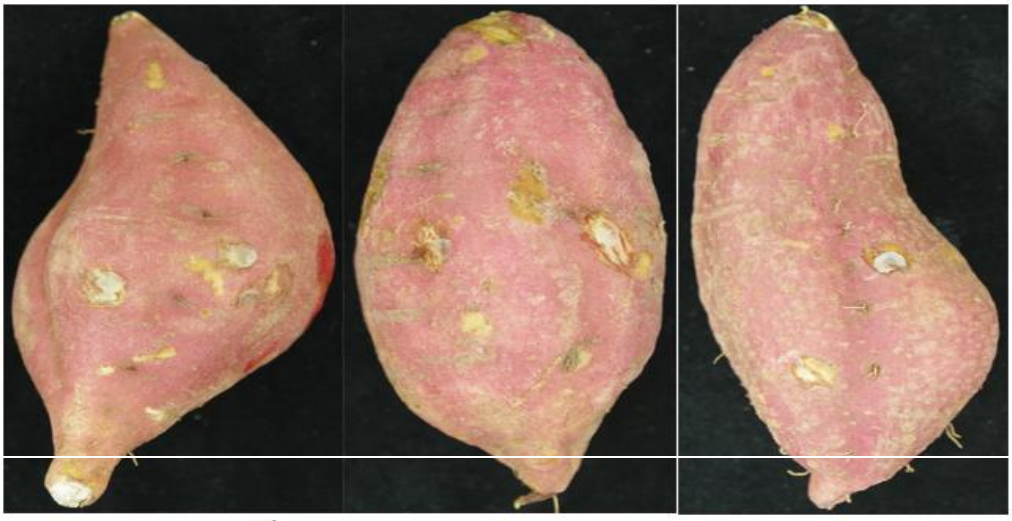


Figure 10.3 Treatment 2

The figures above CK, treatment 1 and treatment 2 shows the sweet potatoes inoculated with Rhizhopus creeping for 10 days. From the 10 days observation the fludioxonile pesticide plus calamus essential oil treatment had a good control and preventive effect on sweet potatoes.

Table3: The situation of sweet potato soft rot disease under different treatments for 2 and 10 days

Time	Treatment	Disease Index	Control Effect（%）
2 days	CK	19.73	—
	Treatment 1	1.95	90.1
	Treatment 2	2.7	86.3
10 days	CK	17.98	—
	Treatment 1	3.01	83.6
	Treatment 2	3.54	80.3

The trial was conducted in April 2023 at the Plant-Fungal Interaction Laboratory of the College of Modern Agriculture, Zhejiang Agricultural and Forestry University, The storage temperature was 16 °C, The humidity was（85±5）%, The sweet potato variety was Tianmu sweet potato.

According to the measurement of disease spot diameter, hyphae grew in the control group at 2 days of treatment, and only rare hyphae growth in treatment 1 and treatment 2, with a control effect of 90.1% and 86.3%, and the control group increased and mycelial infection was more obvious after 2 days of treatment, and the hyphae growth in treatment group 1 and treatment 2 was still very small, and the prevention effect was 83.6% and 80.3%. Conclusion: The combination of fluconitrile and calamus essential oil can effectively prevent and treat soft rot in sweet potato.

6 Discussion

This research gives us a detailed insight on sweet potatoes and one of the main disease affecting them, the sweet potato soft rot disease. Different kinds of experiments mentioned in this paper suggests

the preventive and control methods that can be adopted to manage this disease. In the first chapter an introduction and brief history of the sweet potatoes and soft rot disease has been highlighted, it further described the pesticides and essential oils that were used in this research paper and the research trend. The further chapters give an insight of how control agents and essential oils could possibly manage the disease. First, the fungus was identified by observing the spore morphology and hyphal morphology using a YG10 microscope. Highly effective green prevention and control methods of sweet potato soft rot disease were attempted by screening 16 different kinds of control agents at different concentration (i.e 1 μg/mL, 1.25 μg/mL, 2.5 μg/mL, 5 μg/mL, 20 μg/mL). Results found 3 kinds of fungicides were effective which were Fluazinam, Fludioxonil and Tebuconazole. Another experiment was conducted on 9 different kinds of essential oils (oranges, citronella, garlic, stone calamus, sweet orange, mugwort leaf, sandalwood, *Artemisia* annua, cedar) and results shows that stone calamus, oranges, citronella and garlic had a higher inhibition rate implying that they have better inhibitory effect of the essential oil on the fungus. Furthermore an experiment on sweet potato soft rot inoculation was conducted and results showed that the fludioxonile pesticide plus calamus essential oil treatment had a good preventive and control effect on sweet potatoes.

Sweet potatoes are susceptible to a number of diseases, among which is fungal soft rot of sweet potato. Sweet potato soft rot is caused by the necrotrophic, Zygomycete fungus *Rhizopus stolonifer*. The production of sweet potatoes is accompanied by severe diseases caused by fungus *R. stolonifer* leading to enomrous losses in yield or production and quality worldwide this evidence enough as in to why we should take preventive and control measures to curb the disease. Furthermore it is one of the most common disease of sweet potato soft rot. Rotting of tubers by fungal soft rot during storage differs from 31.3% to 36.8%. *R. stolonifer* is a ubiquitous fungus that also causes postharvest soft rot on several fruits and vegetables, most observed in sweet potatoes and stone-fruits. This soft rot disease has an economic importance storage problem because of their pathogenicity to a wide range of crops from which potatoes are the most important. Rhizopus soft rot typically appears during postharvest handling, transporting and is rarely seen in the field. Symptoms usually originate at a wounded area in the sweet potato and consist of a soft, watery rot that progresses quickly under suitable conditions and can result in full decay of an infected root in few days mostly in three days. White to gray fungal mycelium producing black sporangia are often observed growing on decayed roots. One of the most important methods to control rhizopus soft rot disease in sweet potato globally is the chemical methods, using antibiotics and fungicides. This suggest the reason why our method was used that is testing different kinds of fungicides to know which one is more effective.

Experiments were all conducted in the laboratory from the identification of the fungus to attempting, highly effective green prevention and control methods of sweet potato soft rot disease through screening control agents and essential oils. Sweet potatoes have so many beneficial uses it is therefore important to protect them from any diseases by applying control and preventive measures.

7 Conclusion

In this paper, research was conducted with the aim of identifying which control agent or essential oil has the high inhibition rate or is effective in terms of prevention and control of sweet potato soft rot disease. It was carried out by means of experiments in the laboratory. The experiments mainly included identification of the fungus by observing the spore morphology and hyphal morphorlogy, and attempt highly effective green prevention and control methods of sweet potato soft rot disease using various control agents and essential oils. This research paper also highlighted the importance of sweet potatoes and traced on its history.

Sweet potatoes are susceptible to a number of diseases, among which is fungal soft rot of sweet potato. Sweet potato soft rot is caused by the necrotrophic, Zygomycete fungus *Rhizopus stolonifer*. The production of sweet potatoes is accompanied by severe diseases caused by fungus *R. stolonifer* leading to enomrous losses in yield or production and quality worldwide this evidence enough as in to why we should take preventive and control measures to curb the disease. Furthermore it is one of the most common disease of sweet potato soft rot. Rotting of tubers by fungal soft rot during storage differs from 31.3% to 36.8%. *R. stolonifer* is a fungus that also causes postharvest soft rot on several fruits and vegetables, most observed in sweet potatoes and stone-fruits. This soft rot disease has an economic importance storage problem because of their pathogenicity to a wide range of crops from which potatoes are the most important. Rhizopus soft rot typically appears during postharvest handling, transporting and is rarely seen in the field. Symptoms usually originate at a wounded area in the sweet potato and consist of a soft, watery rot that progresses quickly under suitable conditions and it can result in full decay of an infected root in few days mostly in three days. White to gray fungal mycelium produced black sporangia are often observed on decayed roots. One of the most important methods to control rhizopus soft rot disease in sweet potato globally is the chemical methods, using antibiotics and fungicides.

The identification of the fungus by observing the spore morphology and hyphal morphology was aimed to confirm the causal agent obtained was *R. stolonifer*. Experiments on highly effective green prevention and control methods of sweet potato soft rot disease was aimed to identify which control agents is effective in terms of prevention and control of the sweet potato soft rot disease; on 9 different essential oils, was to explore the effects of these essential oils on the prevention and control of the fungal pathogen. In addition an experiment on sweet potato soft rot inoculation was conducted and results showed that the fludioxonile pesticide plus calamus essential oil treatment had a good preventive and control effect on sweet potatoes.

Soft rot of sweet potato is the main disease of sweet potato during storage. If not controlled, the disease will cause great harm to sweet potatoes, and finally lead to the quality loss and yield losses of sweet potatoes. It is therefore of great benefit and importance to conduct this research was to know if these methods work. If certain control agents are effective, the quality loss and yield losses can be reduced by implementing these methods.

Acknowledgement

I would like to express my gratitude to Zhejiang A & F University for granting me this opportunity of being a student here and fulfilling my dream of studying abroad. I would also like to thank the department of agriculture and food science for the great assistance of using the equipment's and allowing me do my research.

To my supervisor associate professor Dr. Shi Haojie and my colleague Mr. Xuan, thank you so much for your great assistance throughout my research of which it would not have been a success if not for your help. Thank you for your commitment and dedication, you made sure that "no stone was left unturned" went according to plan.

Last but not the least I would also like to express my sincere gratitude to my parents Mr. R. Sameya and Mrs. M. Sameya and the Confucius institute for their support and financial aid.

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