International Journal of Agriculture and Forestry 2013, 3(2): 34-39
DOI: 10.5923/j.ijaf.20130302.02

Aldehydes Compounds for Controlling Black Scurf Disease of Potato (*Solanum Tubrosum* L.) under Field Conditions

Mohamed A. Abd-Alla , Nehal S. El-Mougy, Mokhtar M. Abd-El- Kader*, Farid Abd-El-Kareem, Nadia G. El-Gamal, Ried S. R. El-Mohamedy

Plant Pathology Department, National Research Centre, El-Behouth St. Dokki 12622, Giza, Egypt

Abstract Field trials were carried out for evaluating the efficacy of Acetaldehyde and Benzaldehyde soil application on black scurf disease of potato plants. Laboratory tests revealed that all treatments significantly reduced the linear growth of Rhizoctonia solani. Acetaldehyde and Benzaldehyde solutions at concentrations of 4.0 ml/L completely inhibited the linear growth of Rhizoctonia solani in vitro. Acetaldehyde and Benzaldehyde at concentrations of 5.0 and 10.0 ml/L in addition to fungicide (Basamid) at 50 g/m2 of soil) were applied under field conditions to study their effect on black scurf disease and tuber yield of potato plants. Results indicate that that all treatments significantly reduced the disease incidence of potato plants. The highest reduction was obtained with Acetaldehyde and Benzaldehyde at concentrations of 10.0 ml/L and fungicide Basamid which reduced the disease incidence more than 85.0, 84.2 and 82.9% respectively during two successive growing seasons. Acetaldehyde and Benzaldehyde at concentrations of 5.0 ml/L reduced the black scurf incidence by 72.3 and 68.4% respectively. As for potato yield the highest increased was obtained with Acetaldehyde and Benzaldehyde at concentrations of 10.0 ml/L and fungicide Basamid which increased the tuber yield more than 59.0, 54.4 and 54.4% respectively during two successive growing seasons. Acetaldehyde and Benzaldehyde at concentration of 5.0 ml/L increased the potato yield by 36.5 and 45.0%, respectively.

Keywords Acetaldehyde, Benzaldehyde, Black Scurf Disease, Potato, R. Solani

1. Introduction

Soil borne diseases are still a major threat to potato cultivation in Egypt. Several important potato pathogens in Egypt originated from soil borne inoculums. Potato plants are susceptible to devastation by various diseases such as Black scurf caused by *Rhizoctonia solani* and dry rot caused by *Fusarium sambucinum*[1, 2, 3].

Potato plants (*Solanum tuberosum* L.) considered one of the most important vegetable crops overall the world. Rhizoctonia diseases of potato are caused by the fungus *Rhizoctonia solani* Kühn can be found on all underground parts of the plant at different times during the growing season. *Rhizoctonia solani* causes black scurf on tubers, stem and stolen canker on underground stems and stolens and occurs wherever potatoes are grown. The symptoms of the disease are found on both above and below ground portions of the plant. Black scurf is the most conspicuous sign of Rhizoctonia disease[4, 5]. In this phase of the disease the fungus forms dark brown to black hard masses on the surface of the tuber. These are called sclerotia, the resting bodies of the fungus.

Although black scurf is the most noticeable sign of Rhizoctonia, stem canker, is the most damaging of the disease as it occurs underground and often goes unnoticed[6, 7]. Early in the season, the fungus attacks germinating sprouts underground before emerging shoots above soil surface. The sprout may be killed outright if lesions form near the growing tip. Damage at this stage results in delayed emergence and is expressed as poor and uneven stands with weakened plants[7].

Controlling such disease mainly depend on fungicides treatments[8],but fungicides application cause hazards to human health and increase environmental pollution such as toxic residues and selection of resistant isolates of the pathogen[9, 10, 11]. Therefore, alternative treatments for control of plant diseases are needed. The search for safer phytochemicals with lower environmental and mammalian toxicity is a major concern and imperative[12, 13].

Acetaldehyde and Benzaldehyde produced by fruits during ripening showed antifungal activity. Acetaldehyde has been found effective in postharvest protection of apples,

* Corresponding author:
mokh_nrc@yahoo.com (M.M. Abdel-Kader)
Published online at http://journal.sapub.org/ijaf

sweet cherries and stone fruits[14, 15, 16]. Also, Acetaldehyde has been tested against decay microorganisms commonly found on strawberry fruit such as *Botrytis cinerea* and *Rhizopus stolonifer*[17]. Damage at this stage results in delayed emergence and is expressed as poor and uneven stands with weakened plants[16, 17].

Some plant volatiles, *e.g.* acetaldehyde, Benzaldehyde, Cinnamaldhyde, ethanol, benzyl alcohol, nerolidol, 2-nonanone have also been found to have antifungal activity against the fruit and vegetable pathogens *Penicillium digitatum*, *Rhizopus stolonifer*, *Colletotrichum musae* and *Erwinia caratovora* during *in vitro* trials. Certain plant volatile aldehydes *i.e.* Acetaldehyde has also been reported to inhibit postharvest microorganisms such as *Erwinia carotovora*, *Pseudomonas fluorescens*, *Monilinia fructicola*, *Penicillium* spp. and various species of yeast commonly found on fruit and vegetables[18, 19]. Benzaldehyde has been used fumigating peaches to protect them against Rhizopus rot, and it is also totally inhibited spore germination of *Botrytis cinerea* and germination of *Monilinia fructicola*[16, 18, 19, 20].

The main objectives of the present research were designed to study the efficacy of Acetaldehyde and Benzaldehyde on the mycelial growth of *Rhizoctonia solani* as well as their application as soil treatment against black scurf incidence of potato plants under field conditions and its influence on tuber yield.

2. Materials and Methods

2.1. Pathogen, Plant Material and Chemicals

Different isolates of *Rhizoctonia solani* were isolated from diseased potato tubers samples collected from different fields located at Nubaria region, Behera Governorate, Egypt. This work was done by the authors of the present work. The isolated fungi were tested for their ability to induce Black scurf disease of potato and proved their pathogenicity in this regard. The highly aggressiveness isolate was used in the present study. Potato seeds cv. Diamond were used for cultivation under field conditions. Pure active ingredients of Acetaldehyde and Benzaldehyde (RAMADA Co., Egypt were used. These chemicals were kept in a refrigerator at 5°C till use.

2.2. Laboratory Tests

The inhibitor effect of volatile aldehyde solutions at different concentrations against the linear growth of *Rhizoctonia solani* was evaluated *in vitro*. The aldehydes concentrations of 0.50, 1.0, 2.0 and 4.0 ml/L were tested. A different certain volumes of aldehydes were added to flasks containing sterilized PDA medium before its solidifying to obtained the proposed concentrations, then poured in Petri dishes. Another set Petri dishes containing PDA aldehyde-free medium were kept as control treatment. Disks (6- mm-diameter) taken from the edge of fungus culture were placed in the centre of each Petri plates. Five Petri plates were used as replicates. All Petri plates were incubated at 25 ± 1°C. Fungus linear growth was measured when the control plates reached full growth, then the average diameter of linear growth was calculated and the percentage of reduction in mycelial growth was calculated for aldehydes concentrations relative to the control treatment. This test was repeated three times.

2.3. Field Experiments

Experiments were carried out at the Researches and experimental station (NRC) in Nubaryia region, Behera Governorate, Egypt for two successive growing seasons. The influence of aldehyde solutions application against black scurf disease incidence and potato tuber yield was determined during the two successive growing seasons. Field experiments consisted of plots (4x8 m) each comprised of 8 rows and 32 holes / row was conducted in a Complete Randomized Block design with three replicates (plots) for each particular treatment.

Treatments: Acetaldehyde and Benzaldehyde solutions at two concentrations *i.e.* 5.0 and 10.0 ml/L, in addition to the fungicide (Basamid) at 50 g/m^2 of soil) were applied before potato cultivation.

Application: All plots were irrigated to full water holding capacity. Three days later, the irrigated plots were sprayed with acetaldehyde and Benzaldehyde solutions as well as the fungicide at proposed concentrations at the ratio of 10 L/m^2, then covered with polyethylene sheets for another three days. All plots were cultivated with potato seeds cv. Diamond, three days after polyethylene sheets removal.

Disease assessment and yield determination: Percent of diseased plant was recorded up to 90 days of planting. Tuber yield of potato (kg/m^2) for each treatment was also determined. All the previous procedures were repeated twice at two successive growing seasons at the same area.

2.4. Statistical Analysis

One way analysis of variance (ANOVA) was used to analyze differences between inhibitor concentrations of Acetaldehyde, Benzaldehyde and the linear growth of *R. solani* as well as differences between Acetaldehyde, Benzaldehyde, Basamid and root rot incidence; produced yield at different applied concentrations under laboratory (*in vitro*) and field conditions (*in vivo*). MSTAT-C program (V2.1) was used to perform the analysis of variance. Turkey test for multiple comparisons among means was utilized[21].

3. Results and Discussion

3.1. Laboratory Tests

The effect of increased concentrations of Acetaldehyde and Benzaldehyde on the growth of the fungus *R. solani* is presented in Table (1). Obtained results showed that Acetaldehyde and Benzaldehyde were significantly able to reduce gradually the linear fungal growth by increasing their concentrations. Complete growth inhibition of *R. solani,* was observed at concentration of 4.0 ml/L of both aldehydes tested. Meanwhile, the fungal growth reduction as (39.5, 44.2%); (74.2, 68.2%) and (86.0, 83.5%) was obtained with Acetaldehyde and Benzaldehyde treatment at 0.5, 1.0 and 2.0 ml/L, in respective order.

Table 1. Average linear growth of *Rhizoctonia solani* in response to different concentrations of Acetaldehyde and Benzaldehyde

Aldehydes	Concentrations (ml/L)	Linear growth of *R. solani* (mm)	Growth reduction %
Acetaldehyde	0.50	54.4 b	39.5
	1.00	23.2 c	74.2
	2.00	12.6 d	86.0
	4.00	0.0 e	100
Benzaldehyde	0.50	50.2 b	44.2
	1.00	28.6 c	68.2
	2.00	14.8 d	83.5
	4.00	0.0 e	100
Control		90.0 a	0

Means followed by the same letter are not significantly different at P ≤ 0.05.

Similar report in previous study[22] recorded that aldehydes vapors with low concentrations for 30 min in fumigation chamber significantly inhibited mycelium growth and spore germination of *Botrytis cinerae*. Complete inhibition of linear growth and spore germination was obtained with Acetaldehyde and Cinnamaldhyde vapors at concentrations of 50 and 100 µl/l. Moderate reduction in linear growth and spore germination was obtained with Acetaldehyde and Cinnamaldhyde vapors at concentration of 25 µl/l. As for aldehydes solutions, results indicated that all tested concentrations of Benzaldehyde showed more effective inhibition against *B. cinearea* linear growth and spore germination as compared with other tested plant aldehydes. They suggested that that the use of some plant volatiles aldehydes as a vapors phase and/or liquid phase can be used as alternative of chemical fungicides to controlling gray mold disease of strawberry fruits during transportation, marketing and storage.

In this regards, several workers have demonstrated the ability of Acetaldehyde to inhibit the development of postharvest rots on various crops. Thus, significant reduction of *Botrytis cinerea* and *Rhizopus stolonifer* on strawberries and grapes and of *Penicillium expansum* on apples has been achieved by Acetaldehyde treatment[18, 19]. Moreover, certain plant volatile aldehydes *i.e.* Acetaldehyde, Benzaldehyde and Cinnamaldhyde showed antifungal activity in postharvest protection of apples, sweet cherries and stone fruits[15, 16].

Benzaldehyde, produced by fruit metabolism, also have a fungistatic /fungicidal activity when utilized in postharvest treatment against *Monilinia laxa* and *Rhizopus stolonifer*. Acetaldehyde has been also tested against decay microorganisms commonly found on strawberry fruit such as *B. cinerea* and *Rhizopus stolonifer* and various species of yeast and bacteria commonly found on fruit and vegetables[18, 19].

Also, it was reported that the plant volatiles aldehydes, *i.e.* acetaldehyde, benzaldehyde and cinnamaldhyde were found to be strong inhibitors of fungal growth and shown a germicidal effect when applied at low concentrations[17]. Other plant volatiles aldehydes, *i.e.* Cinnamon bark oil (rich in Cinnamaldhyde) and Cinnamon extracts as well as ethanol, benzyl alcohol, nerolidol, 2-nonanone, â-ionone, and ethyl formate vapors have fungistatic and fungicidal activity against the anthracnose and crown rot pathogens. It was found that spraying them on banana prior to storage controlled crown rot and extended shelf-life. Also they could inhibit the growth of *Rhizopus stolonifer, Penicillium digitatum, Colletotrichum musae, Erwinia carotovora*[16, 19, 20].

The antifungal activities of cinnamaldehyde and eugenol congeners against white-rot fungus *Lenzites betulina* and brown-rot fungus *Laetiporus sulphureus* were evaluated *in vitro*[23]. Results revealed that cinnamaldehyde, a-methyl cinnamaldehyde, (E)-2-methylcinnamic acid, eugenol and isoeugenol exhibited strong antifungal activity against all fungi tested. Furthermore, the presence of the methyl moiety in the ortho position may have a considerable influence on the inhibitory action against *L. betulina* and *L. sulphureus*.

3.2. Field Experiments

Table 2. Effect of some plant aldehydes and Basamid soil treatment on black scurf disease of potato plants under field conditions

Treatment	Concentration	Black scurf incidence (%)				
		First growing season	Reduction %	Second growing season	Reduction %	Average Disease incidence
Acetaldehyde	5ml/L	10.5 b	72.3	10.8 b	73.0	10.6
	10ml/L	5.5 c	85.5	5.0 c	87.5	5.2
Benzaldehyde	5ml/L	12.0 b	68.4	12.2 b	69.5	12.1
	10ml/L	6.0 c	84.2	5.8 c	85.5	5.9
Basamid	50g/m^2	6.5 c	82.8	6.1 c	84.7	6.3
Untreated control		38.0 a	-	40.0 a	-	39.0

Means followed by the same letter are not significantly different at P ≤ 0.05.

In field trials for two successive growing seasons, the efficacy of controlling *R. solani* (Black scurf) on potato using aldehydes and fungicide measures was determined. The results presented in Table (2) showed superior significant effect of aldehydes and fungicide treatments against the incidence of Black scurf disease comparing with control. The average recorded percentage of potato black scurf disease incidence during the two successive growing seasons was 39.0% in untreated soil naturally infested with the pathogen *R. solani*. This percentage sharply decreased down to 10.6, 5.2 and 12.1, 5.9% in the presence of applied Acetaldehyde and Benzaldehyde at 5ml/L and 10ml/L, respectively. Meanwhile 6.3% disease incidence was recorded in applied soil with Basamid (50g/m^2). Moreover, the highest reduction was obtained with Acetaldehyde and Benzaldehyde at 10ml/L and fungicide Basamid which reduced the disease incidence more for about 86.6, 84.8 and 83.3%, respectively during two growing seasons. Meanwhile, Acetaldehyde and Benzaldehyde at 5ml/L showed lower effect as 72.8 and 68.9% disease reduction although it significantly differed than untreated control treatment (Table2).

In the present study an interesting observation was noticed, that reduction in disease incidence reflected positively on the produced yield. In this regards, results in Table (3) indicate that the average highest tuber yield was obtained with Acetaldehyde and Benzaldehyde at 10ml/L and fungicide Basamid which recorded as 3.4, 3.3 and 3.3 Kg/m^2, respectively. Meanwhile, Acetaldehyde and Benzaldehyde were lesser effective for increasing yield that it cause only an increase of 38.0 and 42.8% over control treatment.

Table 3. Effect of soil treatment with aldehydes and Basamid on potato yield under field conditions

Treatment	Concentration	Potato yield (Kg/m^2)				
		First growing season	Increase %	Second growing season	Increase %	Average Yield
Acetaldehyde	5ml/L	3.0 b	36.3	2.9 b	45.0	2.9
	10ml/L	3.5 a	59.0	3.3 a	65.0	3.4
Benzaldehyde	5ml/L	3.2 b	45.4	2.9 b	45.0	3.0
	10ml/L	3.4 a	54.5	3.3 a	65.0	3.3
Basamid	50g/m^2	3.4 a	54.5	3.2 a	60.0	3.3
Untreated control		2.2 c	0	2.0 c	0	2.1

Means followed by the same letter are not significantly different at $P \leq 0.05$.

There are a few cited reports explaining the furfural mode of action against soil microflora. In this respect,[24] successfully used benzaldehyde against soil-borne pathogens, control Fusarium wilt on melons, root rot on strawberries, Phytophthora root rot on citrus, replant syndrome on apples, club rot on cabbage, and damping-off on vegetable crops. Furthermore, Benzaldehyde, have a fungicidal activity when utilized as postharvest treatment against *Monilinia laxa* and *Rhizopus stolonifer*[18, 19, 20]. The fungicidal effect of Acetaldehyde and other aldehydes may be direct or it may be achieved through the augmentation of fruit resistance *via* synthesis of antifungal materials. It has been reported that in oranges, Acetaldehyde is the precursor, *via* acetyl CoA, of mevalonic acid, which is the precursor of all other monoterpenes, including the phytoalexin, limonene[16].

Application of acetaldehyde or an anaerobic treatment with N_2 or CO_2 for 24h, which increased limonene levels, protected orange fruit against fungal decay[19]. They added that acetaldehyde vapor induced the leakage of electrolytes, sugars and amino acids from the fungi *B. cinerea* and *R. stolonifer*, which suggests that cell membranes are irreversibly disrupted by acetaldehyde as a first step towards inhibition of the fungus activity[19]. Furthermore, Benzaldehyde has been used for fumigating table grapes peaches against Rhizopus rot, gray mould and brown rot of stone fruits[14, 19, 20, 24].

Similarly it was reported that[25] cinnamaldehyde is a strong antifungal agent against human pathogens. According to their results, cinnamaldehyde may be a potential lead compound for the development of antifungal drugs through the control b-(1,3)-glucan and chitin synthesis in yeasts and molds[26].

In recent years, some naturally occurring volatile compounds with nematicidal and fungicidal properties have been reported[27, 28]. Some of these compounds stimulated development of populations of fungi and bacteria antagonistic to soilborne pathogens[29].

In this concern a study was conducted to determine the effects of combinations of organic amendments and benzaldehyde on plant-parasitic and non-parasitic nematode populations, soil microbial activity, and plant growth[30]. Benzaldehyde applied alone or in combination with the amendments exerted a selective action on the activity and composition of microbial populations in the soybean rhizosphere. In control soils the bacterial flora was predominantly Gram-negative, while in soils amended with velvetbean or kudzu alone or with benzaldehyde, Gram-positive bacteria were dominant. Mycoflora promoted by the different amendments or combinations with benzaldehyde included species of *Aspergillus*, *Myrothecium*, *Penicillium* and *Trichoderma*. At the end of the experiment population of bacteria were increased by benzaldehyde or its combinations with organic amendments, and selectivity was evident by increases in Gram-negative bacteria including *Burkholderia* spp.

The results with bacteria agree with research conducted by[29, 31] who reported selective pressure of natural aromatic compounds including benzaldehyde, toward Gram-negative bacteria. *Burkholderia* comprises a large and diverse genus which includes species which have been associated with biological control of several soilborne pathogens[32, 33]. Results from this case study illustrated the ability exerted by some organic materials and benzaldehyde to change the composition of a particular soil. It has been shown that some amendments and aromatic compounds may stimulate antagonistic activities against soilborne plant pathogens by inducing changes in taxonomic composition and physiological activities of soil microflora [29, 31, 34].

In general[35] attributed the antimicrobial effects of aromatic compounds to different mechanisms such as: a) increased permeability and loss of cellular constituents as a result of interference with cell membranes, b) impairing of enzyme systems, including those involved in the production of cellular energy and synthesis of structural components, and c) the inactivation or destruction of genetic material.

On the light of the obtained results in the present work, it was found that naturally aromatic compounds may be useful in reducing the need for chemical-based pesticides, increasing desirable soil properties, enhancing natural antagonism, and providing for the proper disposal of agro-industrial wastes.Therefore, it is suggested that aldehydes could be considered as a broad spectrum micro-biocide.

ACKNOWLEDGEMENTS

This work was supported financially by the National Research Centre Fund (NRC), Egypt, Grant No. 9050204.

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