Cairo University

Journal of Advanced Research

ORIGINAL ARTICLE

Efficacy of a pyrimidine derivative to control spot disease on *Solanum melongena* caused by *Alternaria alternata*

Nemat M. Hassan [a], Mohamed I. Abu-Doubara [a], Mohamed A. Waly [b], Mamdouh M. Nemat Alla [a,*]

[a] *Botany Department, Faculty of Science, Damietta University, Egypt*
[b] *Chemistry Department, Faculty of Science, Damietta University, Egypt*

Abstract   The pyrimidine derivative (4,6-dimethyl-N-phenyldiethyl pyrimidine, DPDP) was tested as a foliar spray fungicide at 50 mg l^{-1} for protection of eggplant (*Solanum melongena*) from spot disease caused by *Alternaria alternata*. Varied concentrations of DPDP (10–50 mg l^{-1}) differentially inhibited mycelial growth, conidial count and conidial germination of *A. alternata* growth *in vitro*; the magnitude of inhibition increased with increasing concentration. *In vivo*, an experiment was conducted in pots using a complete block randomized design and repeated twice with three replications and four treatments (control, *A. alternata* alone, DPDP alone and combination of DPDP and *A. alternata*) for 5 weeks (1 plant in pot × 3 pots per set (3 replications per treatment) × 4 sets (4 treatments) × 5 weeks × 2 experimental repetitions = 120 pots). In this experiment, 10-day-old eggplant seedlings were transplanted in pots and then inoculated with *A. alternata*, DPDP or their combination 1 week later. Leaves of the *A. alternata*-infected eggplant suffered from chlorosis, necrosis and brown spots during the subsequent 5 weeks. Disease intensity was obvious in infected leaves but withdrawn by DPDP. There were relationships between incidence and severity, greater in plant leaves infected *A. alternata* alone and diminished with the presence of DPDP. Moreover, the infection resulted in reductions in growth, decreases in contents of anthocyanins, chlorophylls, carotenoids and thiols as well as inhibitions in activities of superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione-S-transferase (GST). Nonetheless, the application of DPDP at 50 mg led to a recovery of the infected eggplant; the infection-induced deleterious effects were mostly reversed by DPDP. However, treatment with DPDP alone seemed with no significant impacts. Due to its safe use to host and the inhibition for the pathogen, DPDP could be suggested as an efficient fungicide for protection of eggplant to control *A. alternata* spot disease.

© 2012 Cairo University. Production and hosting by Elsevier B.V. All rights reserved.

* Corresponding author. Tel.: + 20 57 2400233; fax: + 20 57 2403868.
E-mail address: mamnematalla@mans.edu.eg (M.M. Nemat Alla).

Production and hosting by Elsevier
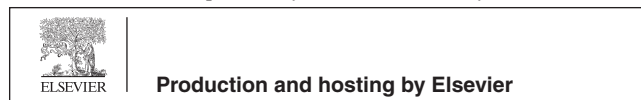
Introduction

Spot disease caused by *Alternaria alternata* is one of the most important diseases for many plants including eggplant (*Solanum melongena*) [1]. The disease appears as dark-brown

necrotic lesions on leaves and fruits that may coalesce to form large necrotic areas. It is attributed to causing leaf necrosis and leaf drop, also causing fruit spotting and rotting during harvest and postharvest stages. Induction of plant defense reactions enhances protection against different types of pathogens and utilizes the plants own defense mechanisms in controlling plant diseases [2]. Several mechanisms that mediate the disease protection include blocking of disease cycle, the direct inhibition of pathogen growth [3] and the induction of resistance to plant against pathogen infection [4]. The most common method for controlling these pathogens is the use of fungicides. On the other hand, the development of resistance in pathogenic fungi to common fungicides and increasing residual hazardous effects on human health and environmental pollution has given a thrust to search for new derivatives that can obstruct the fungal pathogenicity. The fungicide-induced delay of senescence was due to an enhanced antioxidant enzyme activity protecting the plants from harmful reactive oxygen species (ROS) [5]. In response to ROS, plants develop an efficient antioxidative protection system against them [6,7]. Antioxidants play a crucial role in plant defense mechanism against reactive oxygen species [8].

Pyrimidine derivatives exhibit a broad spectrum of biological activities depending on type of the substituent [9]. Of these derivatives, 4,6-dimethyl-N-phenyldiethyl pyrimidine (DPDP) has a wide range of medicinal chemistry as antimicrobial (anti-inflammatory and analgesic) [10] and antifungal [11] activities. Therefore, the present work aims to test the fungicidal effects of this pyrimidine derivative as an effective and safe fungicide for protection of eggplant (S. melongena) from A. alternata causing spot disease.

Material and methods

Chemicals

The pyrimidine derivative (4,6-dimethyl-N-phenyldiethyl pyrimidine, DPDP) was kindly supplied by Dr. El-Ezaby, Chemistry Department, Faculty of Science at Damietta, Mansoura University, Egypt.

Mycelial growth and conidial germination of A. alternata

A. alternata (Fr.) Keissler was kindly supplied by Prof. Dr. Amira A. El-Fallal, Botany Department, Faculty of Science Damietta University, Egypt. Mycelial growth of A. alternata was tested in petri dishes on agar medium containing the following constituents (g l^{-1}): sucrose 20, NaNO$_3$ 2, KH$_2$PO$_4$ 0.5, KCl 0.5, MgSO$_4$.7H$_2$O 0.5, FeSO$_4$.7H$_2$O 0.01, agar 8, and distilled water 1 l. The PH was adjusted to 7. Tests were performed by culturing A. alternata on agar medium containing different concentrations of DPDP (10, 20, 30, 40 and 50 mg l^{-1}) in triplicates. The diameter of the colonies was measured after 7 days from incubation at 30 °C. The inhibition of mycelial growth was calculated as percentage of mycelium growth on medium with DPDP relative to that on medium without DPDP (the control).

For conidial germination, a conidial suspension of A. alternata grown on medium without DPDP was prepared in sterile distilled water and the concentration was adjusted to 1×10^5 conidia per ml. A drop (500 µl) of the conidial suspension

was added to two replicate plates of each DPDP concentration, spread across the plate and partially dried in a laminar flow hood for approximately 20 min. After 4 h of incubation at 30 °C, 100 conidia per each concentration (10, 20, 30, 40 and 50 mg l^{-1}) were examined for their germination under the light microscope. The inhibition of conidial germination was calculated as percentage of germinated conidia on medium with DPDP relative to that on medium without DPDP.

Inhibition zone estimation was carried out by culturing A. alternata on agar medium using the agar disc method. 200 µl of each concentration of DPDP were loaded into holes in the cultured agar medium. The inhibition zone was measured after 7 days from incubation at 30 °C. Moreover, conidial number was counted for each treatment in a similar area and volume.

The experiments of mycelial growth, conidial germination and inhibition zone were repeated twice in triplicate, so that the mean obtained was for six replicates. The research was continued in vivo using the most effective concentration DPDP (50 mg l^{-1}) as a foliar spray fungicide to eggplant for the control of A. alternata causing spot disease.

Plant material and growth conditions

Seeds of eggplant (S. melongena var esculentum) were surface sterilized by immersing in 3% sodium hypochlorite solution for 10 min, thoroughly washed, soaked for 4 h and germinated in loamy/sand soil (1/1, v/v) in pots under the cultivation conditions (14 ± 1 h photoperiod, 450–500 µmol m^{-2} s^{-1} photosynthetic photon flux density, 75–80% relative humidity, and 26 ± 2/16 ± 2 °C day/night schedule) and irrigated daily by water. Ten-day-old seedlings were transplanted in pots (30 cm diameter × 25 cm height), irrigated with water weekly and fertilized with urea only once (5 g per seedling). Each pot contained only one plant. One week later, pots were divided into four sets. An experiment was conducted using a factorial arrangement based on a complete block randomized design with four treatments and three replications for 5 weeks. The experiment was repeated twice so that each treatment was represented by 6 pots. So, pots with seedlings were divided into four sets; the first was used as control, the second for treatment with A. alternata alone, the third for treatment with DPDP alone the fourth for treatment with combination of DPDP and A. alternata. The experimental design was performed for 120 plants (1 plant in pot × 3 pots per set (3 replications per treatment) × 4 sets (4 treatments) × 5 weeks × 2 experimental repetitions = 120 plants in 120 pots).

A. alternata was grown on liquid medium containing the following constituents (g l^{-1}): sucrose 20, NaNO$_3$ 2, KH$_2$PO$_4$ 0.5, KCl 0.5, MgSO$_4$.7H$_2$O 0.5, FeSO$_4$.7H$_2$O 0.01, distilled water 1 liter. The PH was adjusted to 7.0. After incubation for 1 week at 30 °C, the spores and the mycelia were collected by filtration and shaken well. The concentration of the conidial suspension was adjusted to 1×10^5 conidia ml^{-1} using a hemocytometer. About 5 ml of the conidial suspension were foliar sprayed to each individual plant of the A. alternata treatment set in the early morning using an atomizer (an adequate amount to cover the plant leaves). Also 5 ml of DPDP at 50 mg l^{-1} were applied as foliar spray to each individual plant of the DPDP treatment set. For the combination treatment set, individual plants were sprayed with the same concentration of A. alternata followed by DPDP after 8 h. Samples were

harvested just before treatments (zero time) and during the subsequent 5 weeks (at the 2nd, 3rd, 4th and 5th week).

Assessment of disease intensity

Disease intensity was quantified as disease incidence taking values of either 0 or 1 for not diseased or diseased plant leaves, respectively and as disease severity taking values between 0% and 100% leaf area infected for not diseased or completely diseased plant leaves, respectively [12].

Determination of anthocyanins and photosynthetic pigments

Anthocyanins were extracted from plant tissues (5 g obtained from 6 seedlings) in acidic methanol (HCl, 1% v/v). The absorbance was read at 525 nm and 585 nm [13]. The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted in 85% acetone. The absorbance of the clear extract was determined using the spectrophotometric method of Metzener et al. [14].

Determination of thiol contents

Plant samples (5 g) were homogenized in 20 mM EDTA and centrifuged at 12,000g for 15 min. Total thiols were measured in reaction mixture containing 200 mM Tris–HCl (pH 8.2), 10 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) and absolute methanol [15]. The absorbance was read at 412 nm and the quantity of thiol was calculated from the extinction coefficient $E = 13,100$ mM^{-1} cm^{-1}. To determine non-protein thiols, the supernatant was mixed with trichloroacetic acid (50% w/v) and centrifuged at 10,000g for 15 min and the absorbance was read. The protein-bound thiols were calculated by subtracting the non-protein thiols from total thiols.

Assay of glutathione-S-transeferase (GST), glutathione peroxidase (GPX) and superoxide dismutase (SOD)

For extraction of GST, 5 g of plant tissues were homogenized in 100 mM Tris–HCl, pH 7.5, 2 mM EDTA, 14 mM β-mercaptoethanol and 7.5% (w/v) PVPP and centrifuged at 15,000g for 15 min [16]. Assay of GST was performed in 100 mM phosphate, pH 6.5 containing 5 mM GSH and 1 mM chlorodinitrobenzene (CDNB). After an incubation period for an h at 35 °C, the reaction was stopped and the absorbance was measured at 340 nm. The enzyme activity was calculated from the extinction coefficient $E = 9.6$ mM^{-1} cm^{-1} [17]. GPX was extracted in 100 mM Tris–HCl, pH 7.5, 1 mM EDTA and 2 mM DTT and centrifuged at 15,000g for 20 min [18]. The reaction mixture constituted of 100 mM phosphate, pH 7.0, 2% (w/v) Triton X-100, 0.24 U GSR, 1 mM GSH, 0.15 mM NADPH, and 1 mM cumene hydroperoxide. After incubation at 30 °C for 10 min, the rate of NADPH oxidation was measured by monitoring the absorbance at 340 nm for 3 min and calculated from the extinction coefficient $E = 6.2$ mM^{-1} cm^{-1} [19]. SOD extraction wads performed in 50 mM phosphate, pH 7.8, 0.1% (w/v) BSA, 5.5 mM ascorbate, and 8 mM β-mercaptoethanol. SOD activity was assayed by using the photochemical nitroblue tetrazolium (NBT) method in terms of SOD's ability to inhibit reduction of NBT to form formazan by superoxide in 50 mM phosphate, pH 7.8, 9.9 mM L-methionine, 0.057 mM NBT, 0.025% (w/v) Triton X-100, and 0.1 mM riboflavin at 560 nm [20].

Each of the different four treatments was represented in triplicates (3 pots) and the experiment was repeated twice (6 pots per treatment), so that the mean was obtained from six replications ($\pm$ SE). The full data were statistically analyzed for each time point using one-way ANOVA and LSD at $P < 0.05$.

Results

The application of DPDP at different concentrations (10–50 mg l^{-1}) to *A. alternata in vitro* resulted in differential inhibition in growth; the magnitude of inhibition increased with increasing the concentration of DPDP (Fig. 1). Mycelial growth exerted about 79% inhibition by 50 mg relative to 17% by 10 mg. Similarly, DPDP greatly reduced conidial germination; 50 mg resulted in an inhibition of about 92% relative to only 25% caused by 10 mg. The figure clearly indicates that Im50 (effective DPDP concentration that inhibited mycelial growth by about 50%) was less than 33 mg l^{-1} whereas Ic50 (effective DPDP concentration that inhibited conidial germination by about 50%) was about 25 mg l^{-1}.

At the same time, conidial count was highly reduced by DPDP; greater was the reduction as the concentration increased. Both 40 mg and 50 mg DPDP completely prevented conidial formation relative to 36% inhibition by 10 mg (Fig. 2). On the other hand, the inhibitory effect of DPDP on *A. alternata* growth was also detected as a clear zone on agar media. The inhibition zone was larger in higher concentrations; the inhibition zone caused by 50 mg was about fourfold that induced by 10 mg.

The dose–response curve shows that there was a proportional relationship between the DPDP concentration and the percentage of conidial germination inhibition (Fig. 3). This curve indicates that as the time elapsed, conidial germination increased sharply in control. In the presence of DPDP, conid-
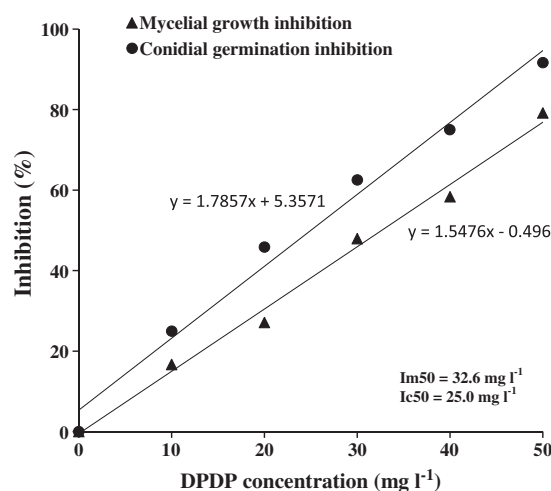

Fig. 1 Effect of 2-amino 4-6-dimethylpyrimidine (DPDP) at different concentrations on growth of *A. alternata in vitro*. Im50 is the effective concentration of DPDP that inhibited mycelial growth by 50%. Ic50 is the effective DPDP concentration that inhibited conidial germination by 50%.
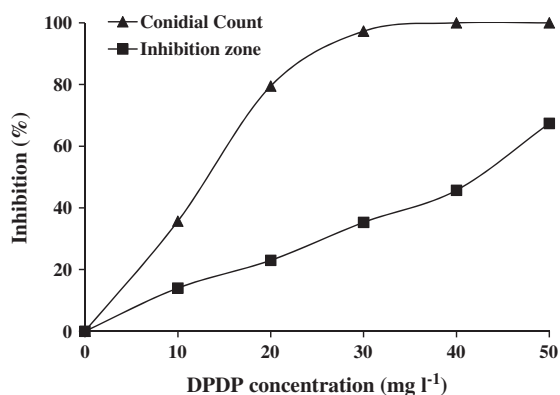
Fig. 2 Effect of 2-amino 4-6-dimethylpyrimidine (DPDP) at different concentrations on growth and conidial germination of *A. alternata in vitro*.
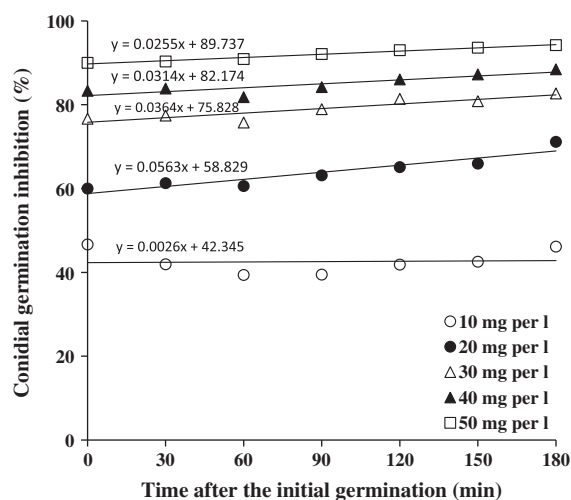

Fig. 3 Time course curve for the conidial germination percentage of *A. alternata* in the presence of 2-amino 4-6-dimethylpyrimidine (DPDP) at different concentrations.

ial germination showed some increases particularly with low concentrations for a few time followed by a diminution, however the highest concentration mostly induced great retardation with time. According to these findings, the *in vivo* application of DPDP was performed with the concentrations 50 mg due to its effectiveness in growth inhibition of *A. alternata in vitro*.

In vivo, symptoms in the fungus infected plant appeared as leaf chlorosis, necrosis and brown spots (Fig. 4). However, the application of DPDP at 50 mg to the infected plants resulted in retreatment of these injury symptoms. It is clear from the figure that disease intensity was obvious in infected leaves but withdrawn by DPDP. Disease incidence and severity increased in leaves infected *A. alternata* alone, nonetheless, the presence of DPDP led to great diminution. In addition, there were relationships between incidence and severity, the magnitudes were greater in plant leaves infected *A. alternata* alone and diminished with the presence of DPDP. So plants treated with such combination appeared as healthy as normal control. On the other hand, the application of DPDP alone to control healthy

plants did not have any deleterious effects; the leaves looked like normal as control.

Moreover, growth parameters of eggplant were greatly affected by the fungus. Fig. 5 shows that *A. alternata* significantly reduced fresh and dry weights of the infected eggplant over the experimental period. By the end of the 5th week, *A. alternata* reduced shoot fresh weight by about 65% and dry weight by about 30%. Nonetheless, the application of DPDP to the infected eggplant overcame growth reductions. When DPDP was present with *A. alternata*, only about 16% reductions were detected in shoot dry weight, whereas shoot fresh weight was mostly not affected.

As shown in Fig. 6, anthocyanins contents were severely inhibited by the fungus during the whole experiment. There were about 57% decreases in anthocyanins content in shoots after 5 weeks of infection with *A. alternata* while the application of DPDP alone resulted in only about 10% reductions. Nevertheless, the application of DPDP to infected plants resulted in increases in anthocyanins contents to reach the control values. The magnitude of reduction in anthocyanins content was retracted by DPDP to become only 12% in shoots. Similarly, the *A. alternata*-infected eggplant showed significant reductions in chlorophyll a, chlorophyll b and carotenoids pigments. After 5 weeks from infection, *A. alternata* resulted in 69%, 58% and 56% reduction in chlorophyll a, chlorophyll b and carotenoids, respectively. However, application of DPDP to the infected plants increased these contents so that these reductions became 4%, 15% and 11%, respectively. Nonetheless, these contents were mostly alike in plants treated with DPDP alone as in control.

In addition, the contents of total-, protein- and nonprotein-thiol were significantly decreased in eggplant shoots by *A. alternata* throughout the experimental period as compared with control values (Fig. 7). About 63%, 67% and 56% reductions in total-, protein- and nonprotein-thiol were induced 5 weeks following infection with *A. alternata*. On the contrary, application of DPDP likely retreated the effects of the fungus. DPDP alone had no effects on thiol contents.

In Fig. 8, the infection resulted in a consistent significant inhibition in the activities of GST, GPX and SOD in shoots. After 5 weeks of infection, GST was inhibited in shoots by 46% while the inhibition reached about 72% and 68% in GPX and SOD, respectively. The magnitude of inhibition in enzyme activities was most likely similar for the different enzymes. However, application of DPDP to the infected plants seemed to overcome the great inhibitions of each enzyme and appeared to reach mostly those of the untreated control. Nevertheless, the application of DPDP alone appeared to cause some inductions in GST and SOD.

Discussion

Mycelial growth of *A. alternata* was greatly inhibited *in vitro* following DPDP application at all concentrations (10–50 mg l^{-1}); 50 mg was the most efficient concentration. It caused the greatest inhibition to mycelial growth and conidial germination. Moreover, it completely prevented the spore formation (conidial count was inhibited by 100%) and induced the greatest inhibition zone. Therefore, it was used *in vivo* as a foliar spray for the protection of eggplant from spot disease caused by *A. alternata*. Time course curve confirmed the
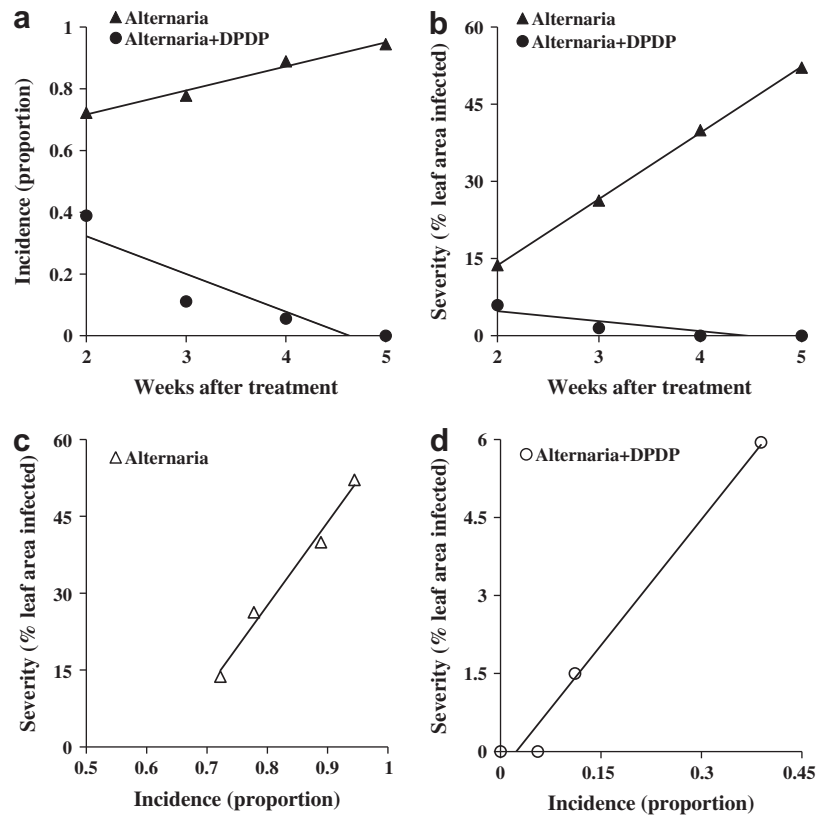
Fig. 4 Disease intensity for eggplant during the subsequent 5 weeks following treatment with *A. alternata* alone or together with 2-amino 4-6-dimethylpyrimidine (DPDP). Application was performed after 1 week of the transplantation of 10-day-old seedlings: (a) disease incidence, (b) disease severity, (c) severity as a function of incidence with *A. alternata* alone and (d) severity as a function of incidence with *A. alternata* together with DPDP. Values are mean of six replications.
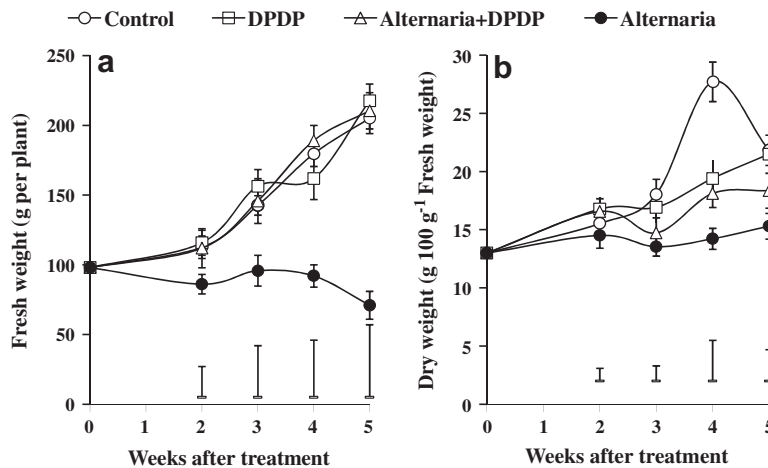

Fig. 5 Effect of *A. alternata* and 2-amino 4-6-dimethylpyrimidine (DPDP) on growth of eggplant during the subsequent 5 weeks. Application was performed after 1 week of the transplantation of 10-day-old seedlings: (a) shoot fresh weight and (b) shoot dry weight. Values are mean ($\pm$SE) of six replications. Vertical bars represent LSD at $p < 0.05$.

efficiency of this concentration upon conidial germination. On the other hand, the symptoms of infection with *A. alternata* appeared as chlorosis, necrosis and brown spots in leaves. Disease intensity was obvious in infected leaves but withdrawn by DPDP. There was an increase in disease incidence and severity by *A. alternata*, however, the presence of DPDP led to great diminution. The relationships between incidence and severity were indicated. Relationships between disease incidence and severity for several plant diseases have been established [12,21,22]. However, these symptoms seemed to be overcome when DPDP was combined with *A. alternata*. Raja et al. [1] recorded leaf spot disease of infected eggplant as
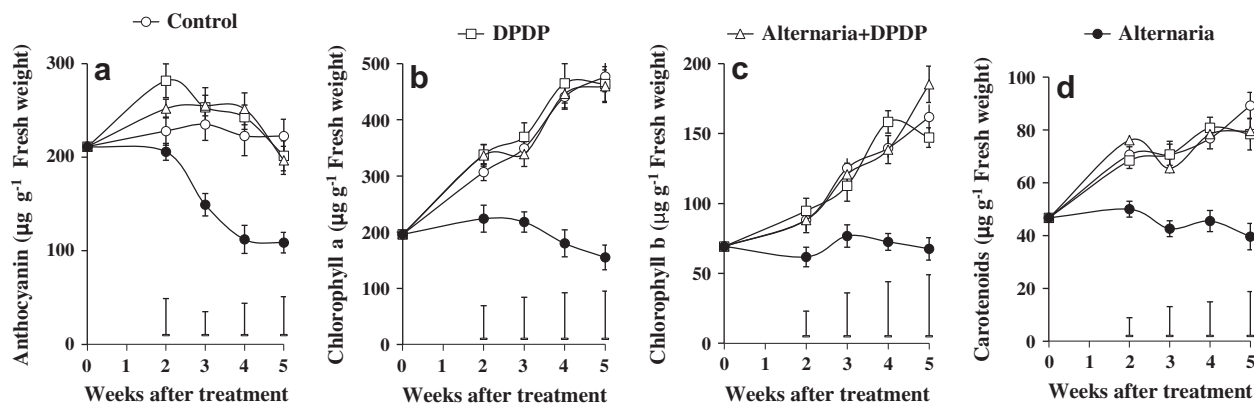
Fig. 6 Effect of *A. alternata* and 2-amino 4-6-dimethylpyrimidine (DPDP) on pigments contents of eggplant during the subsequent 5 weeks. Application was performed after 1 week of the transplantation of 10-day-old seedlings: (a) anthocyanin, (b) chlorophyll a, (c) chlorophyll b and (d) carotenoids. Values are mean ($\pm$ SE) of six replications. Vertical bars represent LSD at $p < 0.05$.
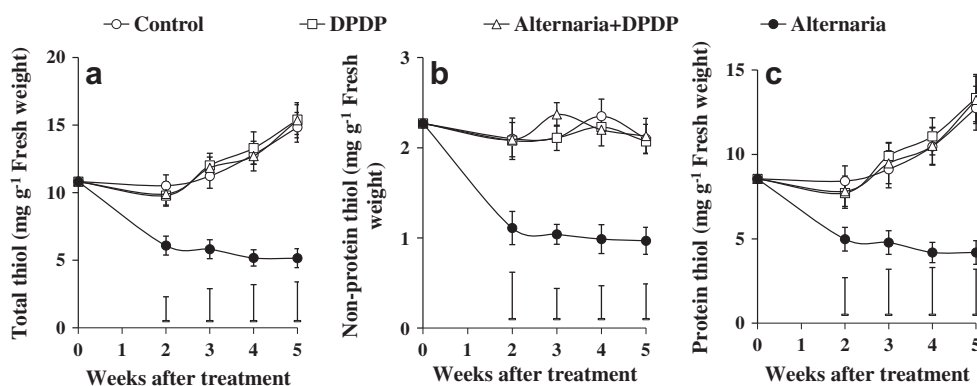

Fig. 7 Effect of *A. alternata* and 2-amino 4-6-dimethylpyrimidine (DPDP) on thiol contents of eggplant during the subsequent 5 weeks. Application was performed after 1 week of the transplantation of 10-day-old seedlings: (a) total thiol, (b) protein thiol and (c) non-protein thiol. Values are mean ($\pm$ SE) of six replications. Vertical bars represent LSD at $p < 0.05$.
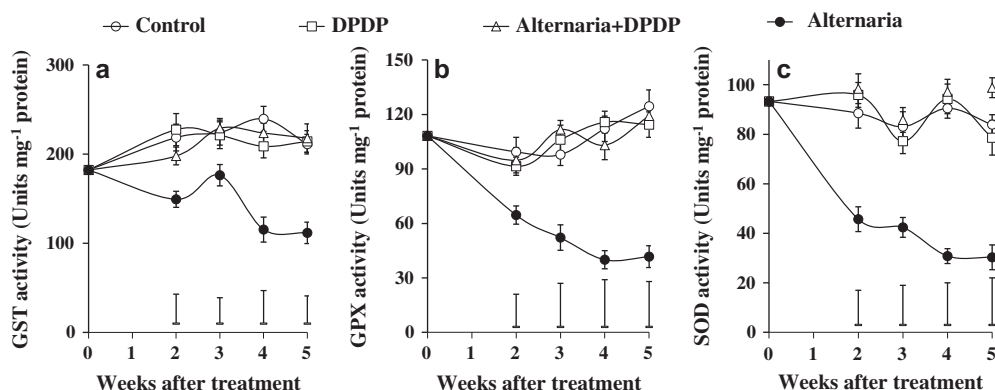

Fig. 8 Effect of *A. alternata* and 2-amino 4-6-dimethylpyrimidine (DPDP) on activities of enzymatic antioxidants of eggplant during the subsequent 5 weeks. Application was performed after 1 week of the transplantation of 10-day-old seedlings: (a) glutathione-S-transeferase (GST), (b) glutathione peroxidase (GPX) and (c) superoxide dismutase (SOD). Values are mean ($\pm$ SE) of six replications. Vertical bars represent LSD at $p < 0.05$.

small, circular and brown necrotic spots all over the foliage. The spots gradually enlarged in size and later became irregular in shape or remained circular with concentric rings or zones. In the later stage of infection, these spots coalesced resulting in

withering, extensive drying and shedding of leaves. The effects of DPDP of the fungus provoked great inhibition in all features of the pathogen growth. These findings could reveal that DPDP led to a recovery of eggplant from spot disease caused

Table 1 Effect of *Alternaria alternata* (*AA*) and 2-amino 4-6-dimethylpyrimidine (DPDP) on eggplant growth, pigments contents, thiol contents, and activities of enzymatic antioxidants of eggplant at the 5th week following treatment. Application of *AA* alone, DPDP alone or their combination was performed after 1 week of the transplantation of 10-day-old seedlings.

	Control	DPDP	DPDP + *AA*	*AA*
Shoot fresh weight (g per plant)	205 ± 11	217 ± 12[a]	210 ± 13[a]	71 ± 10[b]
Shoot dry weight (g 100 g^{-1} fresh weight)	21.8 ± 1.3	21.5 ± 1.1[a]	18.4 ± 1.5[b]	15.3 ± 1.1[b]
Anthocyanin (μg g^{-1} fresh weight)	222 ± 18	201 ± 16[a]	196 ± 15[a]	108 ± 11[b]
Chlorophyll a (μg g^{-1} fresh weight)	477 ± 27	463 ± 31[a]	459 ± 29[a]	155 ± 22[b]
Chlorophyll b (μg g^{-1} fresh weight)	161 ± 9	147 ± 7[a]	185 ± 13[a]	67 ± 8[b]
Carotenoids (μg g^{-1} fresh weight)	89 ± 5	78 ± 4[a]	79 ± 6[a]	39 ± 5[b]
Total thiol (mg g^{-1} fresh weight)	14.8 ± 1.1	15.4 ± 1.1[a]	15.4 ± 1.3[a]	5.2 ± 0.7[b]
Non-protein thiol (mg g^{-1} fresh weight)	2.10 ± 0.16	2.07 ± 0.15[a]	2.13 ± 0.19[a]	0.97 ± 0.15[b]
Protein thiol (mg g^{-1} fresh weight)	12.7 ± 1.3	13.3 ± 1.4[a]	13.2 ± 1.4[a]	4.2 ± 0.7[b]
GST activity (units mg^{-1} protein)	211 ± 11	214 ± 12[a]	217 ± 16[a]	111 ± 12[b]
GPX activity (units mg^{-1} protein)	124 ± 9	114 ± 7[a]	119 ± 7[a]	41 ± 6[b]
SOD activity (units mg^{-1} protein)	83 ± 4	78 ± 7[a]	98 ± 4[a]	30 ± 5[b]

Values are mean (±SE) of six replications and obtained by data interpolation from Figs. 5–8.
GST, glutathione-S-transeferase; GPX, glutathione peroxidase; SOD, superoxide dismutase.
[a] not significantly different.
[b] significantly different from the respective control at $p < 0.05$.

by *A. alternata*. On the other hand, the application of DPDP alone to healthy control plants had no injurious effects. As the retreatment of the infection was not accompanied with negative impacts to host plant, DPDP could be considered as a safe fungicide to protect eggplant from *A. alternata*. Chaerani and Voorrips [23] indicated that *A. alternata* causes diseases to tomato, eggplant and pepper. This disease can be very destructive if left uncontrolled, often resulting complete defoliation.

The present results show that fresh and dry weights of the infected eggplant were reduced by *A. alternata*; however, DPDP appeared to alleviate these reductions. On the other hand, the infection led to significant reductions in chlorophylls, carotenoids and anthocyanins contents in eggplant, nevertheless, such reductions were nullified by DPDP. The decreases in photosynthetic pigments and/or in anthocyanins would result in reduction of plant growth might be due to malfunction of the photosynthetic machinery and/or changes in secondary metabolites used in plant defense against stress. Therefore, DPDP could be used to control *A. alternata* through elevating host resistance and/or inhibiting the pathogen virulence. Nonetheless, the DPDP elevated pigment levels in the infected plants but caused little, if any, changes in control concluding that it is not a stress elicitor.

Plants can defend themselves against diseases caused by different pathogens through a wide variety of mechanisms that may be local or systemic, inducible or constitutive [24]. Antioxidants play a crucial role in plant defense mechanism. Thiols are the supply reserves of glutathione (GSH) which is considered as the most important non-enzymatic antioxidant. It is regarded as a key component of antioxidant defenses in most aerobic organisms [25]. May et al. [26] affirmed that GSH is an abundant and ubiquitous thiol with proposed roles in the storage and transport of reduced sulfur, the synthesis of proteins and nucleic acids and as a modulator of enzyme activity. They concluded that GSH correlates with the tolerance of plants to xenobiotics and to biotic and abiotic stresses. The decreases in thiol pool were obvious in infected plants; nevertheless, DPDP most likely alleviated these effects. In confirmation, thiol-pool functions as a stress indicator and plays several roles in oxidative stress [27,28]. These results support the

importance of thiol for protection of eggplant from oxidative stress induced from infection with *A. alternata*. GSH and GST are very efficient in counteracting the destructive effects of ROS and so retard the programmed cell death. GSH, mediated by GST, conjugates with some xenobiotics leading to their inactivation [29]. The antioxidant metabolism is important in determining the ability of plants to survive under stress conditions and the up regulation of these enzymes would help to reduce the buildup of ROS [30]. Some pesticides can induce increases in antioxidant while some others cannot. In this context, Wang et al. [7] found that chlorothalonil resulted in increases in GSH content, activities of GST and glutathione reductase but such increases were not observed in leaves exposed to carbendazim. In addition, they detected that GST and peroxidase activities were induced by chlorothalonil. They suggest that GSH-dependent pathway plays an important role in the chlorothalonil detoxification but not in the carbendazim detoxification in tomato leaves.

The inhibitions of GST, GPX and SOD activities by *A. alternata* were nullified as DPDP was applied to the infected plants. This would confirm that DPDP overcame the *A. alternata*-induced oxidative stress status. Greenberg [31] stated that during plant–microbe interactions, the production of ROS would lead to programmed cell death and cellular defense against pathogen attack. When produced in excess, ROS can also serve as secondary messengers in the pathogen-response signal transduction pathway [32]. Several mechanisms that mediate the disease protection include blocking of disease cycle, the direct inhibition of pathogen growth [3] and the induction of resistance to plant against pathogen infection [4]. In the present results, the inhibition of mycelial growth of *A. alternata* as well as the count and germination of conidia by DPDP would conclude that both growth and reproduction of the pathogen were greatly affected. Meanwhile, the growth of the host was not negatively influenced by DPDP. Both actions could point to conclude that DPDP might cause an elevation in host resistance and diminution in the pathogen virulence.

Increased resistance could result from secondary metabolites, the phytoalexin precursors, and the non-enzymatic or the enzymatic antioxidants such as SOD, GPX and GST.

SOD catalyses the dismutation of O_2^- with great efficiency in the production of H_2O_2 and O_2 [6,25,29]. The decrease in SOD activity could impair the O_2^- scavenging system of cells; however, increased activity would enhance the production of H_2O_2 which in turn becomes very harmful if left without scavenging [29]. However, peroxidase uses H_2O_2 for regeneration of GSH in its reduced form and therefore is efficient in H_2O_2 breakdown [6]. In addition, GPX appear to play an essential protective role in the scavenging processes when coordinated with SOD activity [8] through scavenging H_2O_2 generated primarily by SOD action. In the present results, SOD was declined by the infection concluding that the host may suffer from oxidative stress with an accumulation of H_2O_2 which, in turn, is used by peroxidase. So, the application of DPDP increased SOD, GPX and GST activities with a consequence of augmenting the production of antioxidants to increase ROS scavenging. As a whole, it is obvious that eggplant was greatly affected by *A. alternata*. The infection resulted in reductions in growth and led to decreases in contents of anthocyanins, photosynthetic pigments and thiols as well as activities of GST, GPX and SOD, the effects augmented with the elapse of time and appeared significant in the 5th week (Table 1). On the contrary, the application of DPDP seemed safe to healthy plants and moreover, overcame the effects of the infection.

In conclusion DPDP inhibited *A. alternata* growth *in vitro* suggesting that this compound might be an efficient fungicide. *In vivo*, leaves of *A. alternata*-infected eggplant suffered from chlorosis, necrosis and brown spots. These symptoms were accompanied with physiological parameters. Disease intensity was obvious due to infection but retracted by DPDP. Both incidence and severity were increased by *A. alternata* but diminished by DPDP. The deleterious effects were overcome by DPDP which seemed to inhibit the pathogen virulence and/or to elevate host resistance. The safety use to eggplant in addition to the inhibition of the pathogen could suggest that it is an efficient fungicide for protection of eggplant from *A. alternata*. Further studies have to be performed for understanding some important issues such as the fungicide selectivity, the exact target site, the recommended field dose, the application time, its biochemical mode of action and its fate in the environment.

Acknowledgements

References

[1] Raja P, Reddy R, Allam US. First report of *Alternaria tenuissima* causing leaf spot and fruit rot on eggplant (*Solanum melongena*) in India. New Dis Rep 2005;12:31.

[2] Van Wees S, Pieterse C, Trijssenaar A, Westende V, Hartog Y, Van Loon L. Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Mol Plant Microb Interact 1997;10:716–24.

[3] Vicentini C, Forlani G, Manfrini M, Romagnoli C, Mares D. Development of new fungicides against *Magnaporthe grisea*: synthesis and biological activity of pyrazolo [3,4-d] [1,3] thiazine, pyrazolo[1,5-c][1,3,5]thiadiazine and pyrazolo [3,4-d] pyrimidine derivatives. J Agric Food Chem 2002;50:4839–45.

[4] Ahn I, Kim S, Lee Y. Vitamin B1 functions as an activator of plant disease resistance. Plant Physiol 2005;138:1505–15.

[5] Zhang Y, Zhang X, Chang-Jun C, Ming-Guo Z, Han-Cheng W. Effects of fungicides JS399-19, azoxystrobin, tebuconazloe, and carbendazim on the physiological and biochemical indices and grain yield of winter wheat. Pestic Biochem Physiol 2010;98:151–7.

[6] Hassan NM, Nemat Alla MM. Oxidative stress in herbicide-treated broad bean and maize plants. Acta Physiol Plant 2005;27:429–38.

[7] Wang J, Jiang Y, Chen S, Xia X, Shi K, Zhou Y, et al. The different responses of glutathione-dependent detoxification pathway to fungicide chlorothalonil and carbendazim in tomato leaves. Chemosphere 2010;79:958–65.

[8] Nemat Alla MM, Badawi A, Hassan NM, El-Bastawisy Z, Badran E. Herbicide tolerance in maize is related increased levels of glutathione and glutathione-associated enzymes. Acta Physiol Plant 2008;30:371–9.

[9] Kumar A, Sudhir S, Prem C. Syntheses of novel antimycobacterial combinatorial libraries of structurally diverse substituted pyrimidines by three-component solid-phase reactions. Bioorg Med Chem Lett 2002;12:667–9.

[10] Sondhi S, Nirupma S, Monika J, Ashok K. Synthesis, anti-inflammatory and analgesic activities evaluation of some mono, bi and tricyclic pyrimidine derivatives. Bioorg Med Chem 2005;136:158–66.

[11] Mangalagiu G, Ungureanu M, Grosu G, Mangalagiu I, Petrovanu M. New pyrrolo-pyrimidine derivatives with antifungal or antibacterial properties *in vitro*. Ann Pharm Fran 2001;59:139–40.

[12] Mcroberts N, Hughes G, Madden LV. The theoretical basis and practical application of relationships between different disease intensity measurements in plants. Ann Appl Biol 2003;142:191–211.

[13] Hoagland R. Effect of glyphosate on metabolism of phenolic compounds. VI. Effect of glyphosine and glyphosate metabolites on phenylalanine ammonia lyase activity, growth and protein, chlorophyll and anthocyanin level in soybean (*Glycine max*) seedlings. Weed Sci 1980;28:393–400.

[14] Metzener H, Rau H, Senger H. Unterschungen zur Synchronisierbarkeit einzelner Pigment Mangel Mutanten von Chlorella. Planta 1965;65:186–94.

[15] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue by Ellman's reagent. Anal Biochem 1968;25:192–208.

[16] Dixon D, Edwards R, Robinson N, Fordham-Skelton A, Cole D. Spectrum of glutathione transferase activities in maize. Brighton Crop Prot Conf – Weeds 1995:255–60.

[17] Askelof P, Guthenberg C, Jakobson I, Mannervik B. Purification and characterization of 2 glutathione-S-transferase activities from rate liver. Biochem J 1975;147:513–22.

[18] Edwards R. Characterization of glutathione transferase and glutathione peroxidases in pea (*Pisum sativum*). Physiol Plant 1996;98:594–604.

[19] Nagalakshmi N, Prasad M. Reponses of glutathione cycle enzymes and glutathione metabolism to copper stress in *Scenedesmus bijugatus*. Plant Sci 2001;160:291–9.

[20] Beyer WF, Fridovich Y. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 1987;161:559–66.

[21] Chuang TY, Jeger MJ. Relationship between incidence and severity of banana leaf spot in Taiwan. Phytopathology 1987;77:1537–41.

[22] McRoberts N, Hughes G, Madden LV. Incorporating spatial variability into disease progress models for crop pathogens. Aspects Appl Biol 1996;46:75–82.

[23] Chaerani R, Voorrips RE. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. J. Gen Plant Path 2006;72:335–47.

[24] Saikia R, Srivastava AK, Singh K, Arora DK. Effect of iron availability on induction systemic resistance to *Fusarium* wilt of chickpea by *Pseudomonas spp*. Mycobiology 2005;33:35–40.

[25] Nemat Alla MM, Hassan NM. Changes of antioxidants and GSH-associated enzymes in isoproturon-treated maize. Acta Physiol Plant 2007;29:247–58.

[26] May M, Vernoux T, Leaver C, Van Montagu M, Inze D. Glutathione homeostasis in plant: implications for environmental sensing and plant development. J Exp Bot 1998;49:649–67.

[27] Nemat Alla MM. Glutathione regulation of glutathione S-transferase and peroxidase activity in herbicide-treated *Zea mays*. Plant Physiol Biochem 1995;33:185–92.

[28] Mendoza-Cózatl DG, Moreno-Sánchez R. Control of glutathione and phytochelatin synthesis under cadmium stress. Pathway modeling for plants. J Theor Biol 2006;238:919–36.

[29] Nemat Alla MM, Hassan NM. Changes of antioxidants levels in two maize lines following atrazine treatments. Plant Physiol Biochem 2006;44:202–10.

[30] Dewir Y, Chakrabarty D, Ali M, Hahn E, Paek K. Lipid peroxidation and antioxidant enzyme activities of *Euphorbia millii* hyperhydric shoots. Environ Exp Bot 2006;58:93–9.

[31] Greenberg JT. Programmed cell death in plant-pathogen interactions. Annu Rev Plant Physiol Plant Mol Biol 1997;48:525–45.

[32] Veal E, Day A, Morgan B. Hydrogen peroxide sensing and signaling. Mol Cell 2007;26:1–14.