

Induction of Resistance in Cauliflower against Alternaria Blight using Potassium and Phosphonic Salts

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ABSTRACT

The effect of exogenous spray application of seven potassium and phosphonic resistance-inducer salts viz. KH_2PO_4 , H_3PO_3 , K_2HPO_4 , K_2SO_4 , salicylic acid (SA), $\text{NH}_4\text{H}_2\text{PO}_4$ and KOH at 0.5, 1.0, 2.0 and 4.0 ml/l dosages to cauliflower (*Brassica oleracea* L. var. *botrytis* subvar. *cauliflora*) leaves on induction of resistance against Alternaria blight (*A. brassicicola*) was studied. The resistance inducers evaluated in terms of incidence and intensity against the disease were found to suppress the disease outbreak to varying levels. The protection achieved was in the order of $\text{KH}_2\text{PO}_4 > \text{H}_3\text{PO}_3 > \text{K}_2\text{HPO}_4 > \text{K}_2\text{SO}_4 > \text{SA} > \text{NH}_4\text{H}_2\text{PO}_4 > \text{KOH}$. It was maximum up to three days of treatment but diluted with time, until 15 days. Smaller lesions of Alternaria blight were produced in the case of KH_2PO_4 (0.232 cm) followed by H_3PO_3 (0.304 cm). The minimum number of lesions of leaf area was found in the case of KH_2PO_4 (1.46/2.5 cm²) followed by K_2HPO_4 (2.03/2.5 cm²). These salt solutions, at the dose tested were not phytotoxic to cauliflower foliage. The technology involving low dose application of salts offers an eco-friendly and cost effective plant protection approach. This study provides preliminary information that may facilitate the standardization of immunization technology, using these potassium and phosphonic salt solutions, for the protection of plants in field or greenhouses.

Keywords: *Alternaria brassicicola*, eco-friendly, KH_2PO_4 , resistance inducer, salicylic acid

INTRODUCTION

Cauliflower (*Brassica oleracea* L. var. *botrytis* subvar. *cauliflora*) is one of the common winter vegetable crops grown throughout the world. The area under its cultivation is 0.77 million hectares (mha) with an annual production of 1.38 million tones in the world. In India, it is grown on an area of 0.3 mha with an annual production of 5.2 million tones (Anonymous 1999). Although farmers have adopted it as a commercial crop, the incidence of diseases, especially Alternaria blight (AB), has become a major constraint to its successful cultivation. The pathogens responsible are *Alternaria brassicae* (Berk.) Sacc. and/or *A. brassicicola* (Schw.) Wilts. Both these pathogens, due to their aggressiveness and seed-borne nature, affect the yield right from seed germination until harvest (Lawrence *et al.* 2008).

AB, in addition to causing leaf spot, premature defoliation and curd deterioration, has been reported to be responsible for a reduction as high as 55.9% of seed yield (Prasad and Vishunavat 2006). The pathogen survives in the infected seeds for many years in cool and dry conditions (Maude and Humpherson-Jones 1980).

AB has been managed through chemical fungicides but this approach is not encouraged on account of the residual effect of the applied chemicals in the edible portion (curd) of the crop. The economic reasons too do not support the use of chemicals. There is, thus, a need for an alternative strategy for disease management that must be ecologically viable, economically sound and user-friendly. Recently, a novel concept of disease management through disease-resistance-inducer salts has emerged (Kuč 1982; Reuveni and Reuveni 1997; Vallad and Goodman 2004; da Rocha and Hammerschmidt 2005), which can be made a useful component of the integrated disease management (IDM) in conjugation with other cultural and biological measures. These compounds are applied at low doses and have no direct action on pathogens and act by inducing resistance in

plants (pre-disposition). Additionally, they provide an advantage of stimulating plant growth and no phytotoxic symptoms are observed on the crop following application of these compounds.

Potassium and phosphonic salts have been found to be potential inducers of disease resistance in a number of host-pathogen combinations. These compounds include KH_2PO_4 , K_2HPO_4 , KOH, $\text{NH}_4\text{H}_2\text{PO}_4$ and H_3PO_3 . Application of phosphate salts have been reported to induce systemically acquired host resistance (SAR) in the case of cucumber-*Colletotrichum lagenarium* (Gottstein and Kuć 1989), broad bean-*Uromyces faba* (Walters and Murray 1992), maize-*Puccinia sorghi* (Reuveni *et al.* 1994, 1996a), cucumber-*Sphaerotheca fuliginea* (Reuveni *et al.* 1996b, 1997), rice-*Pyricularia oryzae* (Mandhar *et al.* 1998), cucumber-*Colletotrichum lagenarium* (Orobar *et al.* 2002) and barley-*Erysiphe polygoni* (Mitchell and Walters 2004) pathosystems. Furthermore, Panicker and Ganadharan (1999) reported that phosphonic acid was able to control downy mildew (*Peronospora sorghi*) of maize. These results, however, contrast with those of Moragrega *et al.* (1998), who demonstrated that phosphonate derivatives only weakly controlled bacterial blast of pear (*Pseudomonas syringae* pv. *syringae*), while the studies of Ouimette and Coffey (1989) proved that monoethyl, dimethyl, and diethyl phosphonate and potassium hypophosphate could efficiently protect *Persea indica* and pepper from *Phytophthora citricola* and *P. capsici*, respectively.

In the present study, evaluation of potential resistance-inducer salts was undertaken and their optimum dose of application was determined. The study also focused on whether the resistance-inducer treatments can be considered for an integrated approach for disease management system for AB of cauliflower.

Table 1 List of disease resistance inducers tested against *Alternaria* blight of cauliflower.

Chemical name	Formula	Company
Ammonium dihydrogen-ortho-phosphate (anhydrous)	NH ₄ H ₂ PO ₄	S.D. Fine Chemical Ltd., Biosar (India)
Potassium dihydrogen-ortho-phosphate	KH ₂ PO ₄	New India Chemical Enterprise, Cochin (India)
Dipotassium hydrogen-ortho phosphate	K ₂ HPO ₄	New India Chemical Enterprise, Cochin
Potassium hydroxide	KOH	E. Merck (India) Ltd., Worli, Bombay (India)
Phosphoric acid	H ₃ PO ₃	Sarabhai M. Chemicals Ltd. Wadiwali (India)
Potassium sulphate	K ₂ SO ₄	S.D. Fine chemical Ltd., Biosar (India)
Salicylic acid	C ₂ H ₆ (OH).COOH	Central Drug House (New Delhi) (p) Ltd. (India)

MATERIALS AND METHODS

Plant material

Cauliflower seeds (cv. 'PG-26') were sown in the first week of October on raised beds. 30-day-old seedlings were used in the experiment.

Disease resistance inducers

Seven known resistance-inducer salts were evaluated for their efficacy in inducing resistance in cauliflower to AB. Their chemical names and other attributes are presented in **Table 1**.

Isolation, purification and maintenance of pathogen culture

The pathogen, *Alternaria brassicicola*, was isolated on potato dextrose agar (PDA). Tiny bits of the leaves having infected portion along with some healthy part were cut and surface sterilized with 0.1% mercuric chloride for 30 sec, followed by repeated washings in sterilized distilled water to remove all traces of the disinfectant. The sterilized bits were transferred to Petri dishes containing distilled water agar (DWA) medium. The Petri dishes were incubated at 25 ± 1°C. The freshly radiating mycelium from the bits was transferred to fresh PDA slants, aseptically. The culture so obtained was purified on PDA. The culture was stored in the refrigerator (set at 4°C) and sub-cultured every month on fresh PDA slants.

Pathogenicity tests

Inoculum preparation

The six-day-old culture was inundated in a Petri dish with sterile distilled water and then rubbed with a curved glass rod. Conidia of the pathogen were brought in solution. The concentration of the suspended conidia was determined using a haemocytometer and adjusted to 5 × 10⁵ conidia ml⁻¹.

Inoculation

Pathogenicity tests were conducted on the leaves of potted cauliflower seedlings (45 days old). The leaves were swabbed with a cotton swab and spray-inoculated using standard inoculum of conidial suspension. The inoculated seedlings were covered with water-sprayed perforated polythene bags for 48 h to ensure proper relative humidity and maintained on glasshouse benches (20-25°C). The uninoculated seedlings that were sprayed with sterilized distilled water served as control. The inoculated leaves were examined regularly for the appearance of symptoms.

Dose-response analysis

To determine effective concentration providing adequate protection against AB, the salts (listed in **Table 1**) were tested at four different doses, viz. 0.5, 1.0, 2.0 and 4.0 ml/l under glasshouse conditions for their ability to induce resistance in cauliflower against the test disease. Each treatment was triplicated in a completely randomized block design (CRBD). The salt solutions in distilled water were sprayed till drip on host seedlings with the help of an atomizer. The seedlings sprayed with water served as control. Data on size (cm) and distribution (number/2.5 cm²) of lesions were recorded in treated leaves and compared with checks.

Assessment of lesion distribution and disease rating

To examine whether the seven test resistance-inducers namely, K₂HPO₄, K₂SO₄, KH₂PO₄, NH₄H₂PO₄, salicylic acid (SA), H₃PO₃ and KOH at four dosages (0.5, 1.0, 2.0 and 4.0 ml/l) exerted any influence on the density of lesions of AB, the number of lesions/2.5 cm² of the leaf lamina were recorded in each case. The distribution of AB lesion was measured by following a simple and easy-to-use technique that involved counting the number of lesions randomly under a 2.5 cm² window made in an aluminium foil and lesion diameter in mm with the help of a ruler.

The observations on percent disease control (PDC) in each treatment were calculated as per the formula given by Yadav *et al.* (1980):

$$PDC = 100[(D_c - D_t) / (D_c)]^{-1}$$

where

D_c = disease intensity in untreated check

D_t = disease intensity in treatment

The time course study was conducted to determine the duration of protection against the disease by inoculating the host at three-day intervals.

Statistical analysis

All the experimental results were subjected to CPSC 1 computer-based statistical analysis software. Data on percentage were transformed to arcsine and analysis of ANOVA was carried out with transformed values. The means were compared for significance (p=0.05).

RESULTS

Data on the effect of different resistance-inducers treatments on the development of AB was studied in terms of its lesion density per unit area of leaf lamina and the expansion of lesions (lesion size) and compared with the untreated check. The data, on the effect of seven test salts each with four dosages, on the progress of test diseases under glass house, are presented in **Tables 2** and **3**.

Effect on development of *Alternaria* blight

All the test salts suppressed the outbreak of the disease to varying levels (**Fig. 1**), and protection was in the order: KH₂PO₄ > H₃PO₃ > K₂HPO₄ > K₂SO₄ > SA > NH₄H₂PO₄ > KOH. Further, the protection was maximum up to three days of treatment that got diluted with the passage of time until up to 15 days, although the level of protection remained much more than the untreated check (**Fig. 1**).

Lesion expansion

Lesion size varied significantly with the treatment of the cauliflower foliage with the test salt (**Table 2**). Bigger lesions (0.378 cm) resulted in the case of KOH followed by 0.368, 0.346, 0.323, 0.331 cm of K₂SO₄, SA, K₂HPO₄ and NH₄H₂PO₄, respectively, which were statistically at par with each other but significantly bigger than 0.232 cm in the case of KH₂PO₄ (**Table 2**). All the treatments produced lesions significantly smaller than the untreated control (0.866 cm). The small sized lesions on leaves receiving the chemical treatment are in fact desired, as the lesions would reduce the photosynthetic efficiency of the host and lead to lesser secondary infections from smaller lesions.

Table 2 Effect of chemical resistance inducers on the size of *Alternaria* blight lesions on cauliflower leaves.

Chemical/salt	Mean lesion diameter (cm) / window (2.5 cm ²)				
	Dose (ml/l)				Mean
	0.5	1.0	2.0	4.0	
KH ₂ PO ₄	0.383 ± 0.083*	0.293 ± 0.007	0.253 ± 0.006	0.000 ± 0.000	0.232 ± 0.164
H ₃ PO ₃	0.421 ± 0.091	0.407 ± 0.005	0.220 ± 0.004	0.167 ± 0.002	0.304 ± 0.129
K ₂ SO ₄	0.507 ± 0.109	0.467 ± 0.002	0.293 ± 0.005	0.206 ± 0.006	0.368 ± 0.142
KOH	0.563 ± 0.122	0.467 ± 0.004	0.307 ± 0.002	0.173 ± 0.003	0.378 ± 0.172
NH ₄ H ₂ PO ₄	0.356 ± 0.077	0.360 ± 0.002	0.320 ± 0.003	0.287 ± 0.004	0.331 ± 0.034
K ₂ HPO ₄	0.548 ± 0.119	0.333 ± 0.004	0.253 ± 0.001	0.159 ± 0.004	0.323 ± 0.165
SA	0.445 ± 0.096	0.380 ± 0.003	0.298 ± 0.002	0.260 ± 0.008	0.346 ± 0.082
Mean	0.460 ± 0.080	0.386 ± 0.065	0.277 ± 0.036	0.178 ± 0.092	
Control	0.866 ± 0.04				

CD (p=0.05) Salt = 0.38; Dose = 0.27; Salt x Dose = 0.77

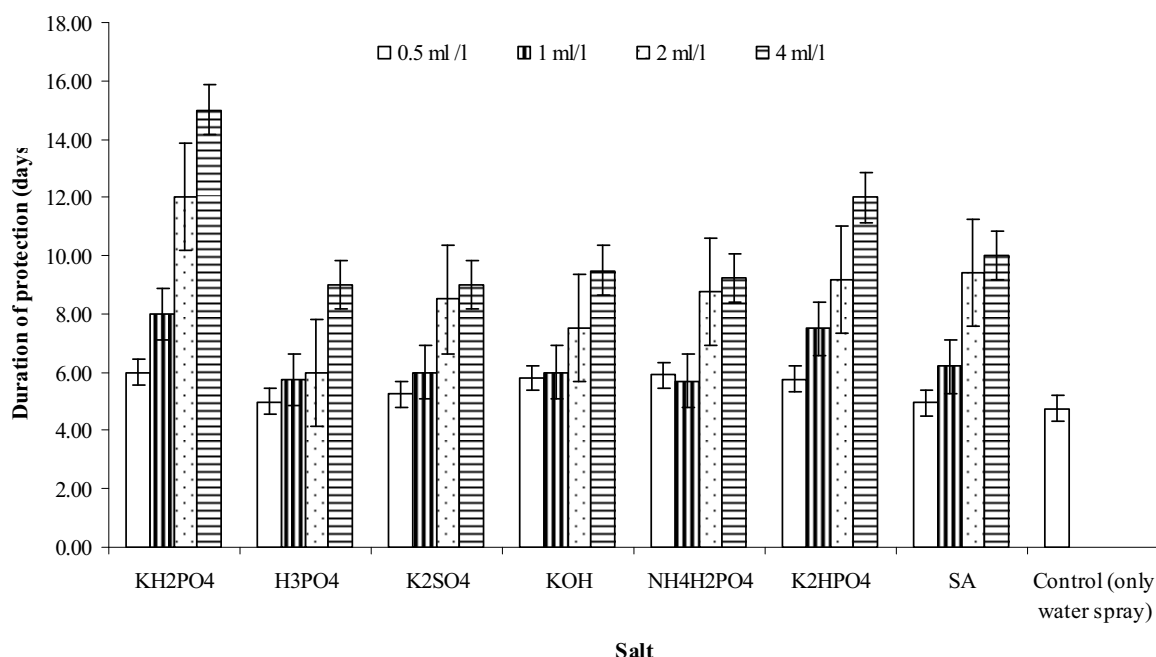
* Values after ± = S.Em

Table 3 Effect of chemical resistance inducers on density of *Alternaria* blight lesion on cauliflower leaves.

Chemical/salt	Mean lesion diameter (cm) / window (2.5 cm ²)				
	Dose (ml/l)				Mean
	0.5	1.0	2.0	4.0	
KH ₂ PO ₄	2.70 ± 0.04*	1.97 ± 0.08	1.17 ± 0.05	0.00 ± 0.00	1.46 ± 1.15
H ₃ PO ₃	3.77 ± 0.03	2.48 ± 0.04	2.62 ± 0.02	1.70 ± 0.01	2.64 ± 0.85
K ₂ SO ₄	5.17 ± 0.03	4.24 ± 0.03	2.40 ± 0.04	1.23 ± 0.04	3.26 ± 1.77
KOH	3.92 ± 0.06	3.50 ± 0.04	2.85 ± 0.05	2.00 ± 0.01	3.07 ± 0.83
NH ₄ H ₂ PO ₄	4.77 ± 0.05	3.27 ± 0.06	2.87 ± 0.03	1.67 ± 0.02	3.15 ± 1.27
K ₂ HPO ₄	2.97 ± 0.01	2.25 ± 0.07	1.73 ± 0.02	1.18 ± 0.03	2.03 ± 0.76
SA	4.55 ± 0.10	3.17 ± 0.02	2.37 ± 0.04	1.48 ± 0.07	2.89 ± 1.30
Mean	3.98 ± 0.91	2.98 ± 0.79	2.29 ± 0.62	1.32 ± 0.64	
Control	6.98 ± 0.87				

CD (p=0.05) Salt = 0.38; Dose = 0.28; Salt x Dose = 0.75

* Values after ± = S.Em

**Fig. 1** Protection of cauliflower against *Alternaria* blight by spray application of salt/chemical at different doses.

The mean lesion size of AB as influenced by the dosage of salt treatment was 0.460, 0.386, 0.277 and 0.178 cm for 0.5, 1.0, 2.0 and 4.0 ml/l of the chemical applied respectively (Table 2). Thus the increased dose of the salt progressively resulted in decreased lesion size. The effect of treatment, dosage and their interaction were statistically significant (Table 2). The suppression of lesion expansion by the chemical treatments is a new dimension in relation to disease development that can have practical implications for devising disease management strategies. Replacement of ecologically harmful fungicides with the phosphonic salts for disease management would be desirable as, unlike fun-

gicides acting deadly on the pathogen, the salts result in the activation of host defense mechanism. Salts being used in low dosage will reduce the plant protection cost as well.

Lesion density

The number of lesions/2.5 cm² of the leaf varied significantly with the test chemical inducer. The maximum number of 3.26 lesions/2.5 cm² was recorded in the case of K₂SO₄ followed by 3.15, 3.07, 2.89, 2.64, 2.03 and 1.46 in the case of NH₄H₂PO₄, KOH, SA, H₃PO₃, K₂HPO₄ and KH₂PO₄, respectively (Table 3). These values were much less than the lesion density in the case of the untreated con-

trol (6.98 lesions/2.5 cm²). The mean lesion density of *Alternaria* blight in different dosages was 3.98, 2.98, 2.29 and 1.32 for 0.5, 1.0, 2.0 and 4.0 ml/l, respectively. Thus the dose of the chemical exerted a retarding effect on lesion density.

No symptoms of toxicity whatsoever were recorded at these dosages in the case of any of the seven treatments. The lesion suppression efficacy varied significantly with the salt, dosage and their interaction (**Table 3**).

DISCUSSION

The aim of the study was to evaluate and quantify the effect of various potassium and phosphonic salts on induction of resistance in cauliflower to AB. The study confirms that these salts induce resistance in cauliflower against the pathogen, and that the induced resistance (IR) is dose dependent. Similar findings have been reported by various workers that phosphate and potassium salts such as K₂HPO₄ or KH₂PO₄ affect conidia production of *S. fuliginea*, *S. pannosa* var. *rosae*, *Puccinia sorghi*, *Leveillula taurica* and induce systemic resistance in the respective host plants such as cucumber, rose, maize and pepper. Becot *et al.* (2000) demonstrated that Phytogard[®], containing 42% K₂HPO₃ provided complete protection (no sporulation) of cauliflower seedlings against *Peronospora parasitica* with concentrations of 7.0 ml/l or higher. It was also shown that the induced resistance was not systemic and lasted at least for 15 days after treatment.

The effect of various concentrations of foliar salt application has not been previously studied using *Alternaria brassicicola*-cauliflower patho-systems. Reveni *et al.* (1996a) observed reduction in the number of lesions and their size from 51% (KH₂PO₄) to 69% (K₂HPO₄) and from 73% (KNO₃) to 91% (K₂HPO₄), respectively as compared with water sprayed plants in maize-*Puccinia sorghi* System. While working on the different host pathogen systems in the same year, Reveni *et al.* (1996b) also found that foliar single applications of K₂HPO₄ and KH₂PO₄ at pH 4.5 or 9.3, KNO₃, KCl, K₂SO₄ and NH₄H₂PO₄ at concentrations ranging from 20 to 100 mM were more effective at significantly reducing *S. fuliginea* on cucumber. In further experiments, Reveni *et al.* (1996b) found that foliar applications of 25 mM of K₂HPO₄ or KH₂PO₄ on a 7- or 14-day schedule were highly effective in controlling natural infection of cucumber powdery mildew. A single application of resistance inducer salts *viz.* K₂HPO₄, K₂SO₄, KH₂PO₄, NH₄H₂PO₄, SA, H₃PO₃ and KOH at four dosages (0.5, 1.0, 2.0 and 4.0 ml/l) gave similar results and provided protection from 3 to 15 days, in the present study. Similar reports on the effect of SA on induction of systemic resistance were made by Mills and Wood (1984) in cucumber against *Colletotrichum lagenarium*, Weete (1992) in sickle pod against *Alternaria cassiae* and Frey and Carver (1998) in pea against *Erysiphe pisi*. The percentage disease reduction over control increased with the time lag given in the inoculum application. The production and transmission of signals by the resistance inducer salt requires a time lag, before which plant fail to respond against the invasion by the pathogen. Our study indicated that a time lag of at least five days between treatment and infection of the pathogen is required for the expression of induced resistance in untreated leaves (**Fig. 1**). Frey and Carver (1998) too emphasized the requirement of a time lag for complete expression of resistance in different host-pathogen systems.

The mode of action of the foliar resistance inducer applications was not studied in our experiments. Treatments may have been effective because of osmotic potential, pH or ion specific effects. The effectiveness of foliar resistance-inducer salts in controlling AB pathogens gives an environmentally friendly option for disease control, and if used in combination with other disease control options, these could be useful in reducing fungicide use or preventing fungicide-insensitive isolates of pathogens from increasing. Reveni and Reveni (1998) used 1% (w/v) KH₂PO₄ alternatively

with fungicides on nectarine trees to control *Sphaerotheca pannosa* and were able to reduce the application by 50%. No evidence of phytotoxicity at the concentrations used was noticed.

In conclusion, our results show, for the first time, that several potassium and phosphonic salts (K₂HPO₄, K₂SO₄, KH₂PO₄, NH₄H₂PO₄, SA, H₃PO₃ and KOH) at various concentrations ranging from 0.5 to 4.0 ml/l resulted in the reduction of growth and development of AB in terms of lesion size and density on cauliflower seedlings under glass house conditions. The disease showed varying levels of suppression through these treatments. Among these resistance inducers KH₂PO₄ is of particular interest because it is effective even at lowest concentration of 0.5 ml/l for up to 6 days, which further provide much better protection even up to 15 days without any symptom expression with 4.0 ml/l concentration after inoculation of *A. brassicicola*, while remaining resistance inducer salts *viz.* K₂HPO₄, K₂SO₄, NH₄H₂PO₄, SA, H₃PO₃ and KOH display efficacy only up to 9 to 12 days with highest concentration without showing any visible symptoms of disease on foliage. The results of this study advance the possibility of using these salts to control AB on cauliflower. However, further work is necessary with the salts to evaluate their efficacy against AB pathogen in several cauliflower cultivars in order to establish whether salt application could eventually be integrated into disease control strategies for cauliflower. In addition, it is also important to evaluate the effect of salt application on the quality of the cauliflower curd harvested for the treated crop.

From the study, it can be inferred that resistance can be induced in susceptible cauliflower seedlings by treatment with chemical resistance inducers. Cauliflower seedlings do respond to induction of resistance against *Alternaria* spp. A time lag of days is required for complete expression of resistance throughout the plant. However, further studies should focus on the molecular mechanisms involved in the release of elicitors, path in signal transduction and production of pathogenesis related proteins (PR-proteins) involved in the defense reaction for *Alternaria* spp., which may pave the way for identifying an appropriate mode of usage of resistance inducers.

ACKNOWLEDGEMENTS

The authors thank to Dr. Karnail Singh, Statistician, Department of Plant Breeding Genetics and Biotechnology for his help in data interpretation in light of statistical analysis and for providing useful inputs for discussion.

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