JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2016. Vol. **10**(1), p. 605-612

Effect of Non-conventional Chemicals and Synthetic Fungicide on Biochemical Characteristics of Chilli Against Fruit Rot Pathogen *Colletotrichum capsici*

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https://doi.org/10.22207/JPAM.10.1.82

(Received: 15 December 2015; accepted: 30 January 2016)

A pot experiment was conducted during 2013-14 at Chaudhary Charan Singh, Haryana Agricultural University, Hisar to evaluate the effect of non-conventional chemical viz., salicylic acid, zinc sulphate, magnesium sulphate, indole acetic acid, indole butyric acid and fungicide viz., carbendazim, on total phenol, flavonol, tannin and electrolyte leakage of red fruits of chilli varieties (susceptible- Pusa Jwala and resistant- Sadabahar) against *Colletotrichum capsici* the causal agent of fruit rot in chilli. The phenol content was increased significantly in both the varieties (resistant and susceptible) when sprayed with salicylic acid followed by pathogen as compared to other non-conventional chemicals at 24 and 48 hours intervals. The increase in total phenol was more pronounced in resistant variety (7.84 mg/g fresh weight) after 48 hours, when sprayed with salicylic acid at 5mM concentration. Flavonol content was higher in uninoculated red fruits of susceptible variety (1.50 mg/g fresh weight) as compared to resistant (1.37 mg/g fresh weight). Tannin content was higher in resistant (3.71 mg/g fresh weight) as well as susceptible (3.09 mg/g fresh weight) varieties after inoculation than uninoculated varieties (1.38 and 1.02 mg/g fresh weight, respectively) at 5mM concentration. The activity of electrolytes was more pronounced in resistant variety as compared to susceptible variety when sprayed with non-conventional and fungicide in all the concentration. Electrolytes leakage was more when sprayed with fungicide as compared with salicylic acid 5mM concentration after 48 hours of pathogen inoculation.

Keywords: Chilli, *Colletotrichum capsici*, Electrolyte leakage, Flavonol, Non-conventional, Phenol, Resistant, Salicylic acid and Tannin.

Among the major diseases of chilli, fruit rot caused by *Colletotrichum capsici* is one of the major diseases in the production of chilli. The disease causes severe damage on red chilli fruits. Host plant resistance is considered as most practical, feasible and an economical method of plant disease management. Defense strategies of plants against pathogens are several, including the production of antifungal chemicals, which are either pre-formed (i.e. already present in plant tissue in different amounts) or induced following infection (e.g. de novo synthesized phytoalexins) (Grayer and Kokubun, 2001). Plants are frequently exposed to various biotic and abiotic stresses and therefore have evolved a multi-layered system of defence mechanisms (Eckey-Kaltenbach *et al.*, 1993). Plant cells respond to these biotic and abiotic stimuli by synthesizing a number of secondary

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metabolites which may protect them against the causal agents. Phenolic phyto-anticipins that inhibit the growth of fungi may include simple phenols, phenolic acids, flavonols, and some isoflavones. Phytoalexins that are induced in response to fungal infection include isoflavonoids, pterocarpans, furocoumarins, flavans, stilbenes, phenanthrenes (Chérif *et al.*, 2007). The accumulation of secondary metabolites especially phenolic compounds can restrict the spread of the pathogen by the formation of biopolymers in plants (e.g. lignin and callose). However, this type of response is only one part of the diverse layers of plant response to pathogen infection. Soluble as well as cell wall-bound phenolic compounds accumulate early after infection in many plant-pathogen systems in both susceptible and resistant interactions. Phenolic compounds can assist in preventing ROS damage by scavengers and protect cells from free radicals (Torres *et al.*, 2006). A number of phenols are regarded as pre-infection inhibitors, providing plants with a certain degree of basic resistance against pathogenic microorganisms (Satisha *et al.*, 2008). External stimuli can modulate the synthesis of phenolic compounds and therefore change the chemical composition or quantities of phenolic compounds in the plants. External stimuli include microbial infections, UV light, mechanical wounding of the plant (Matsuki, 1996), as well as insecticides and herbicides (Daniel *et al.*, 1999). Fungicides as like, maneb, benomyl, and nabam induced the synthesis of hydroxyphaseollin in soybean (Lydon and Duke, 1993). Plant phytohormones such as abscisic acid, jasmonic acid, ethylene and salicylic acid (SA) are important components of different signalling pathways involved in plant defense (War *et al.*, 2011). Plants treated with zinc sulphate 10^{-5} mmol and subsequently challenged with the Sclerotinia stem rot, caused maximum accumulation of tannic, gallic and chlorogenic acids after 24, 48 and 72 h, respectively (Sarma *et al.*, 2007). In plants, flavonoids play an important role in biological processes. Beside their function as pigments in flowers and fruits, to attract pollinators and seed dispersers, flavonoids are involved in UV-scavenging, fertility and disease resistance (Schijlen *et al.*, 2004).

MATERIALS AND METHODS

A pot experiment was conducted at Department of Plant Pathology, CCS, HAU, Hisar, Haryana, India during 2013-14. In this study, two different varieties Pusa Jwala (susceptible to *Colletotrichum capsici*) and Sadabahar (resistant to *Colletotrichum capsici*) of chilli (*Capsicum annuum* L.) were raised in February, 2013 at nursery level and transplanted the seedlings in first week of April. Furthermore, five non-conventional chemicals viz; salicylic acid, zinc sulphate, magnesium sulphate, indole acetic acid, indole butyric acid and and synthetic fungicides viz; carbendazim were sprayed on red fruits of both chilli varieties for biochemical analysis using three different concentrations (0.2 mM, 1 mM and 5 mM). The red fruits (5 fruits on each plant) were then inoculated with standard spore suspension (3×10^4 spore/ml) from 8 days old culture of *Colletotrichum capsici* by pin prick method after 24 hours of chemicals spray. The red fruits sprayed with water and pathogen alone served as control. After the inoculation of pathogen, red fruits were collected at 24 and 48 hours and various biochemical parameters viz; total phenol, flavonol, tannin and electrolyte leakage were studied.

Extraction of phenolic compounds

For extraction and estimation of total phenols, method of Swain and Hills, 1959, was adopted. Weighed 1g of chilli red fruits and ground it with a mortar and pestle in 10ml of 80 per cent alcohol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The clear supernatant was taken and residue was re-extracted thrice with 5ml of 80 per cent alcohol. The supernatant was pooled and final volume was made to 20ml with 80 per cent alcohol).

Estimation of total phenols and flavonol

Total phenols and flavonols in extracted samples were estimated as described by Swain and Hills, 1959. Standard curve was prepared using different concentrations of catechol and tannic acid for total phenol and flavonols respectively.

Estimation of Tannin

Tannin was estimated in extracted samples by using Vanillin-HCl reagent (Burns, 1971). Absorbance was measured at 525nm, and the tannin content was calculated with the help of standard curve of tannic acid.

Estimation of Electrolytic leakage

Electrolytic leakage was estimated by the method described by Mahadevan and Shridhar, 1982. The conductance of the leachates was determined with a digital conductivity meter and expressed as µ mhos.

RESULTS AND DISCUSSION

Total phenol

Total phenol content was increased in resistant as well as susceptible variety when sprayed with non-conventional chemicals and fungicide followed by pathogen inoculation in all the concentration. The increase in phenol content was significantly higher in both the varieties along with pathogen when sprayed with salicylic acid followed by pathogen as compared to other non-conventional chemicals at both intervals. The increase in total phenol was more pronounced in resistant variety (7.84 mg/g fresh weight) after 48 hours, when sprayed with salicylic acid at 5mM concentration. No significant difference was observed in salicylic acid and fungicide spray at 5mM concentration (Table 1).

The importance of phenolic compounds in disease resistance has been recognized since the off-quoted works of Walker (1923, 1926) who demonstrated the protective role of preformed phenolics in onion against smudge pathogen *Colletotrichum circinans.* The resistant onion variety contains protocatechuic acid and catechol. These phenols are water soluble and diffuse from the dead cell layers of the seeds into the infection drop and due to their high toxicity against *Colletotrichum circinans* inhibit germination and penetration (Walker and Link, 1935). Some reports showed the enhancement in total phenol

Table 1. Total phenol gradient (mg/g fresh weight) in the fruits of resistant (Sadabahar) and susceptible variety (Pusa jwala) of chilli in response to non-conventional chemicals followed by pathogen inoculation at different intervals

Chemicals	Concentration (mM)	Pusa jwala Intervals after pathogen inoculation		Mean	Sadabahar Intervals after pathogen inoculation		Mean
		24h	48h		24h	48h	
Salicylic acid	0.2	3.64	4.45	4.05	4.35	5.85	5.10
	1	4.06	4.75	4.41	5.31	7.30	6.31
	5	5.37	6.48	5.93	6.20	7.84	7.02
Zinc sulphate	0.2	2.97	3.42	3.20	4.15	5.45	4.80
	1	3.42	3.85	3.64	4.39	5.65	5.02
	5	4.06	4.22	4.14	4.95	5.70	5.33
Magnesium sulphate	0.2	2.61	3.24	2.93	3.95	5.35	4.65
	1	2.93	3.70	3.32	4.10	5.45	4.78
	5	3.10	3.96	3.53	4.25	5.85	5.05
Indole acetic acid	0.2	3.18	4.03	3.61	4.18	5.60	4.89
	1	3.21	4.12	3.67	4.58	5.75	5.17
	5	3.33	5.00	4.17	4.95	5.95	5.45
Indole butyric acid	0.2	3.60	4.25	3.93	4.25	5.84	5.05
	1	3.80	4.65	4.23	4.35	5.98	5.17
	5	4.25	4.95	4.60	4.60	6.05	5.33
Carbendazim	0.2	4.20	5.11	4.66	5.65	5.85	5.75
	1	4.65	5.23	4.94	5.85	6.35	6.10
	5	4.95	5.30	5.13	6.19	7.86	7.03
Water spray	–	2.75	3.25	3.00	3.65	4.85	4.25
Pathogen spray	–	3.00	4.15	3.58	4.05	5.25	4.65
C.D. (p=0.05)	Varieties (A) 0.06	Time (B) 0.05		Chemicals (C) 0.02	Concentration (D) 0.01		Interaction (A×B×C×D) 0.15

contents in response to both the test pathogen isolates of *Ascochyta rabiei* in resistant genotype E 100Y while it was decreased in susceptible genotype H 208 when subjected to inoculation at 2-10 days intervals (Khirbat and Jalali, 1997). In the present study the total phenol content was increased in resistant as well as susceptible variety when sprayed with non-conventional chemicals and followed by pathogen inoculation. The increase in phenol content was significantly higher in both the red fruits of the variety when sprayed with salicylic acid as compared to other non-conventional chemicals at both intervals. The increase in total phenol was more pronounced in resistant variety when sprayed with salicylic acid at 5mM concentration. No significant difference was observed in case of salicylic acid and fungicide followed by pathogen inoculation at 5mM concentration in both varieties. The total phenol content was increased when groundnut leaves were sprayed with salicylic acid 24 hours before pathogen inoculation (Meena *et al.*, 2001).

Flavonol

Flavonol content was higher in uninoculated red fruits of susceptible variety (1.50 mg/g fresh weight) as compared to resistant (1.37 mg/g fresh weight). There was increase in flavonol content when inoculated with non-conventional chemicals and fungicides followed by 24 and 48 hours after pathogen inoculation. The increase in flavonol content was non-significant between the time intervals (Table 2).

Previous reports showed that high concentrations of flavonoids and alkaloids in the infected plant make the plant resistant to sooty mold of olive leaves (Ilias *et al.*, 2015). The present investigation failed to throw any significant light on its role in fruit rot resistance in chilli. The flavonol

Table 2. Total flavonol gradient (mg/g fresh weight) in the fruits of resistant (Sadabahar) and susceptible variety (Pusa jwala) of chilli in response to non-conventional chemicals followed by pathogen inoculation at different intervals

Chemicals	Concentration (mM)	Pusa jwala Intervals after pathogen inoculation		Mean	Sadabahar Intervals after pathogen inoculation		Mean
		24h	48h		24h	48h	
Salicylic acid	0.2	1.53	1.63	1.58	1.63	1.65	1.64
	1	1.90	2.05	1.97	2.03	2.05	2.04
	5	2.50	2.58	2.54	2.65	2.67	2.65
Zinc sulphate	0.2	1.22	1.31	1.27	1.47	1.50	1.48
	1	1.58	1.61	1.59	1.74	1.79	1.76
	5	2.06	2.10	2.08	2.15	2.19	2.17
Magnesium sulphate	0.2	1.17	1.20	1.18	1.37	1.39	1.37
	1	1.54	1.63	1.58	1.62	1.64	1.63
	5	1.97	2.02	2.00	1.90	1.98	1.94
Indole acetic acid	0.2	1.41	1.47	1.44	1.54	1.56	1.55
	1	1.77	1.83	1.80	1.83	1.84	1.84
	5	1.98	2.01	2.00	2.15	2.22	2.18
Indole butyric acid	0.2	1.47	1.56	1.51	1.58	1.60	1.59
	1	1.81	2.01	1.91	1.92	1.94	1.93
	5	2.01	2.14	2.08	2.33	2.40	2.36
Carbendazim	0.2	1.67	1.71	1.69	1.42	1.51	1.46
	1	1.51	1.59	1.55	1.58	1.76	1.67
	5	1.65	1.76	1.70	1.81	1.95	1.88
Water spray	-	1.42	1.50	1.46	1.25	1.37	1.31
Pathogen spray	-	1.43	1.49	1.46	1.46	1.69	1.58
C.D. (p=0.05)	Varieties (A) 0.01	Time (B) NS		Chemicals (C) 0.02	Concentration (D) 0.03		Interaction (A×B×C×D) 0.05

content was more in red fruits of susceptible variety before inoculation. There was increase in flavonol content when inoculated with non-conventional chemicals and fungicide followed by 24 and 48 hours after pathogen inoculation. The increase in flavonol content was non-significant between time intervals. The flavonol level on challenge inoculation indicated the insignificant role in disease resistance.

Tannin

Total tannin content was higher in resistant (3.71 mg/g fresh weight) as well as susceptible (3.09 mg/g fresh weight) varieties after inoculation than uninoculated varieties. Increase was significantly higher in both the varieties when sprayed with salicylic acid followed by pathogen inoculation than other non-conventional chemicals and fungicide (Table 3). Tannin has been implicated sometimes in disease resistance and sometimes in disease susceptibility. Plants treated with Zinc sulphate 10^{-5} mmol and subsequently challenged with the Sclerotinia stem rot, caused maximum accumulation of tannic, gallic and chlorogenic acids after 24, 48 and 72 h, respectively (Sarma *et al.*, 2007). In the present study the tannin was higher in resistant as well as susceptible variety upon inoculation with *Colletotrichum capsici* than control. Increase was significantly higher in both the varieties when sprayed with salicylic acid followed by pathogen inoculation than other non-conventional chemicals.

Electrolyte leakage

There was significant increase in the activity of electrolytes in resistant variety as compared to susceptible variety when sprayed with non-conventional and fungicide in all the concentration. Increase was more pronounced in resistant variety (30.95) at 5mM concentration

Table 3. Total tannin gradient (mg/g fresh weight) in the fruits of resistant (Sadabahar) and susceptible variety (Pusa jwala) of chilli in response to non-conventional chemicals followed by pathogen inoculation at different intervals

Chemicals	Concentration (mM)	Pusa jwala Intervals after pathogen inoculation		Mean	Sadabahar Intervals after pathogen inoculation		Mean
		24h	48h		24h	48h	
Salicylic acid	0.2	1.82	2.22	2.02	2.51	2.90	2.70
	1	2.22	2.55	2.38	2.87	2.99	2.93
	5	2.69	3.09	2.89	3.05	3.71	3.38
Zinc sulphate	0.2	0.94	1.16	1.05	1.27	1.64	1.45
	1	1.05	1.49	1.27	1.75	1.94	1.85
	5	1.35	1.96	1.65	1.93	2.27	2.10
Magnesium sulphate	0.2	0.80	0.98	0.89	0.98	1.16	1.07
	1	0.98	1.31	1.15	1.35	1.45	1.40
	5	1.16	1.55	1.36	1.53	1.94	1.74
Indole acetic acid	0.2	1.16	1.46	1.31	1.64	1.85	1.75
	1	1.31	1.76	1.54	1.96	2.19	2.07
	5	1.96	2.29	2.12	2.40	2.87	2.64
Indole butyric acid	0.2	1.57	2.01	1.79	2.29	2.39	2.34
	1	1.82	2.34	2.08	2.25	2.64	2.45
	5	2.07	2.69	2.38	2.87	3.38	3.12
Carbendazim	0.2	1.05	1.60	1.33	1.38	1.82	1.60
	1	1.49	1.93	1.71	2.47	2.51	2.49
	5	1.71	2.58	2.15	2.73	2.98	2.86
Water spray	-	0.80	1.02	0.91	1.24	1.38	1.31
Pathogen spray	-	1.75	2.33	2.04	2.22	2.69	2.46
C.D. (p=0.05)	Varieties (A) 0.04	Time (B) 0.04		Chemicals (C) 0.07	Concentration (D) 0.05		Interaction (A×B×C×D) 0.23

when sprayed with salicylic acid as compared to susceptible one. The leakage of electrolyte was more when sprayed with fungicide as compared with salicylic acid 5mM concentration after 48 hours of pathogen inoculation (Table 4).

Change in membrane permeability is the first detectable event in the onset of disease caused by different pathogens. In the present studies, it was found that there was significant increase in the leakage of electrolytes in resistant variety as compared to susceptible variety. Increase was more pronounced in resistant variety as compared to susceptible one with salicylic acid spray at 5mM concentration. The leakage of electrolytes was more when sprayed with fungicide as compared with salicylic acid 5mM concentration after 48 hours of pathogen inoculation. Treatment of leaves of *Cicer arietinum* with Azoxystrobin resulted in electrolyte leakage as measured by increased electrical conductivity (EC). The increase in EC was pronounced with the increase in fungicide concentration and incubation period. The negative EC values obtained in the Difenoconazole treatment may be due to fast and efficient uptake of the fungicide from the ambient solution by the leaf tissue (Nithyameenakshi *et al*., 2006). It is known that along with the electrolyte, leakage of phenol also takes place. One of the probable reason may be the phenol while in contact with oxidizing enzyme get converted into the quinines or higher molecular weight compound which block the cell membrane pore and then decreased the outward flow of electrolytes. Alternatively, the electrolytes themselves get depleted as a result of outward flow from the cells. (Khirbat and Jalali, 2000) while assessing chickpea (*Ascochyta rabiei*) interaction

Table 4. Electrolytic leakage (μ mhos/g fresh weight) in the fruits of resistant (Sadabahar) and susceptible variety (Pusa jwala) of chilli in response to non-conventional chemicals followed by pathogen inoculation at different intervals

Chemicals	Concentration (mM)	Pusa jwala Intervals after pathogen inoculation		Mean	Sadabahar Intervals after pathogen inoculation		Mean
		24h	48h		24h	48h	
Salicylic acid	0.2	10.59	11.10	10.85	20.25	26.10	23.17
	1	11.09	12.10	11.60	21.15	28.85	25.00
	5	12.44	12.99	12.71	23.85	30.95	27.40
Zinc sulphate	0.2	9.15	10.05	9.60	18.20	23.15	20.68
	1	9.50	10.50	10.00	18.95	23.64	21.30
	5	10.42	10.88	10.05	19.65	24.58	22.12
Magnesium sulphate	0.2	9.25	9.54	9.40	18.18	22.75	20.48
	1	9.12	10.58	9.85	18.68	23.18	20.93
	5	9.76	10.93	10.35	19.14	23.52	21.33
Indole acetic acid	0.2	9.85	10.75	10.30	18.66	23.64	21.15
	1	10.15	11.01	10.58	19.12	24.18	21.65
	5	10.34	11.10	10.72	19.96	24.8	22.38
Indole butyric acid	0.2	9.45	10.25	9.85	18.78	24.45	21.50
	1	9.70	10.55	10.13	19.32	25.04	22.18
	5	10.25	10.96	10.61	20.65	25.85	23.25
Carbendazim	0.2	10.80	11.15	10.97	20.60	26.46	23.53
	1	11.25	11.78	11.52	22.45	30.00	26.23
	5	13.05	13.92	13.49	25.30	32.22	28.76
Water spray	-	8.68	9.25	8.97	12.45	14.85	13.65
Pathogen spray	-	9.75	11.95	10.85	17.20	22.78	19.99

C.D. (p=0.05)	Varieties (A)	Time (B)	Chemicals (C)	Concentration (D)	Interaction (A×B×C×D)
	0.02	0.03	0.01	0.01	0.11

reported significant increase in the activity of electrolytes after inoculation with both blight isolates in resistant and susceptible genotypes as compared to uninoculated control.

CONCLUSION

This biochemical study indicated that there was pronounced increase in total phenol and tannin content in resistant variety (Sadabahar) when sprayed with salicylic acid at 5mM concentration. The activity of electrolytes was more in resistant variety as compared to susceptible with salicyclic acid at 5mM concentration. However, there was no significant change in flavonol content after spraying with chemicals.

ACKNOWLEDGEMENTS

The first author sincerely acknowledges ICAR for the financial support in the form of Junior Research Fellowship for M.Sc. research programme. Thanks are also due to the Head and Guide for providing field and laboratory facilities at the Department of plant pathology, CCS HAU, Hisar during the course of this investigation.

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