Int. J. LifeSc. Bt & Pharm. Res. 2012

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 1, No. 3, July 2012

Research Paper

EFFECT OF CATECHOL, GALLIC ACID AND PYROGALLIC ACID ON THE GERMINATION, SEEDLING GROWTH AND THE LEVEL OF ENDOGENOUS PHENOLICS IN CUCUMBER (*CUCUMIS SATIVUS* L.)

Shafiqa Muzaffar[1], Barket Ali[1*] and Niyaz Ahmad Wani[1]

Corresponding Author:* ***Barket Ali,* ✉ *barket_ali5@yahoo.com;*

Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, which are of great significance in plant metabolism. With an objective to study the impact of some of these compounds on the seed germination and seedling growth, the seeds of cucumber (*Cucumis sativus* L.) were treated with distilled water (control), 5×10^{-5} M, 10^{-4} M, 5×10^{-4} M or 10^{-3} M, each of catechol, gallic acid or pyrogallic acid. Treatment of the seeds with the phenolic compounds significantly decreased the germination percentage, growth of radicle and hypocotyls and the fresh and dry weight of the seedlings. Out of the different concentrations of these compounds used, 10^{-3}M caused the maximum inhibition. Among the phenolics used, pyrogallic acid was the inhibitoriest. However, total phenolic content in the seedlings increased in response to different treatments, in a concentration dependent manner.

Keywords: Cucumber, Germination, Growth, Phenolics

INTRODUCTION

Phenolic compounds are of ubiquitous occurrence in plants, which collectively comprise several thousand different chemical structures characterized by hydroxylated aromatic ring(s). They constitute an important component of higher plants and are predominantly found in a wide range of commonly consumed plant parts such as fruits, vegetables, cereals and legumes, and in beverages of plant origin, such as wine, tea and coffee (Cheynier, 2005; and Manach *et al.*, 2004). These compounds are secondary metabolites of plants generally involved in defense against ultraviolet radiation or aggression by pathogens. Several thousands of phenolic compounds have been described in plants and can be grouped into different classes according to their basic chemical structure (such as type

---

[1] Govt. College for Women, M A Road, Srinagar, Jammu and Kashmir.

and number of phenol rings), and into different subclasses, according to specific substitutions in the basic structure, association with carbohydrates and polymerized forms (Manach *et al.,* 2004).

Most of these compounds have received considerable attention as potentially protective factors against human chronic degenerative diseases (cataracts, mascular degeneration, neurodegenerative diseases, and diabetes mellitus), cancer and cardiovascular disease (Scalbert *et al.*, 2005). However, these compounds are also of great significance in plant development. They are involved in diverse processes such as rhizogenesis (Curir *et al.*, 1990), vitrification (Kevers *et al.*, 1984), resistance to biotic and abiotic stress (Delalonde *et al.*, 1996), and redox reactions in soils (Takalama and Oniki, 1992). Additionally, they serve as flower pigments, act as constitutive protection agents against invading organisms, function as signal molecules, act as allelopathic compounds, and affect cell and plant growth (Ndakidemi and Dakora, 2003). They have also been recognized as allelochemicals for weed control (Putnam and Tang, 1986) and plant defence molecules (Vidhyasekaran, 1988). The synthesis and release of phenolics are induced by various biotic and abiotic factors. Boron deficiency can induce the accumulation of phenolics in plants. Tissue injury, pathogen attack, herbivory, and infection by microsymbionts such as rhizobium can also cause synthesis and release of phenolics (Lawson *et al.*, 1996). Inside and outside plant tissues, these metabolites are known to function as phytoalexins, phytoanticipins and node gene inducers (Dakora and Phillips, 2002). Some of the phenolic compounds play important role in several physiological responses in plants, e.g. salicylic acid has a direct involvement in plant growth, thermogenesis, flower induction and uptake of ions. It also affects ethylene biosynthesis, stomatal movement and reverses the effects of ABA on leaf abscission. Enhancement of the level of chlorophyll and carotenoid pigments, photosynthetic rate and modifying the activity of some of the important enzymes are other roles assigned to this and its structurly related phenolic compounds (Hayat *et al.*, 2007).

Present research was conducted with cucumber (*Cucumis sativus* L.) to investigate the impact of exogenous application of catechol, gallic acid and pyrogallic acid on its germination, seedling growth and total phenolic contents in the seedlings.

MATERIALS AND METHODS

Seeds of cucumber (*Cucumis sativus* L. cv. Long Green) were germinated in petridishes containing blotting paper moistened with distilled water (control), 5×10^{-5} M, 10^{-4} M, 5×10^{-4} M or 10^{-3} M of catechol, gallic acid or pyrogallic acid. Each petridishes contained 25 seeds and the petridishes were maintained in dark at 25^0C. The seeds were kept, moist with solutions comprising the respective treatment. Germination (emergence of radicle) of seeds were recorded after every 24 h of soaking. After 72 h of soaking, the lengths of radicle and hypocotyl was measured in 10 seedlings pickedup randomly. The seedlings were weighed to determine their fresh weight and then washed with running tap water followed by washing with distilled water. The seedlings were gently blotted dry with blotting paper and then dryed at 70^0C for 24 h in an oven. After drying, dry weight of the seedlings were recorded. The dry material also used for the estimation of total phenols. Each treatment was replicated thrice and the Least Significant

Difference (LSD) was calculated manually at 5% level to determine the degree of response to various treatments.

RESULTS AND DISCUSSION

The treatment of the seeds with the phenolic compounds viz catechol, gallic acid and pyrogallic acid significantly decreased the germination percentage (Table 1). At 24 h stage of sampling, germination was observed only in the control plants. However, at 48 h and 72 h, a concomitant decrease in % germination with increasing concentration of the phenolic compounds were noticed. The highest concentration i.e., 10^{-3} M of all the phenolics used was the most inhibitory in its impact. Comparing the three compounds, pyrogallic acid caused the maximum inhibition, particularly at 10^{-3}M concentration. This treatment reduced the percentage of germination by 89%, and 90% compared to the control, at 48 h and 72 h, respectively.

The growth of radicle and hypocotyl was supressed with an increase in the concentration each of catechol, gallic acid and pyrogallic acid (Table 2). 5×10^{-5} M concentration of phenolic compounds had least inhibitory impact. However, the degree of inhibition increased with increasing concentration and reached to its maximum level at the concentration 10^{-3} M, irrespective of the phenolic compound used. Likewise, the fresh and dry weight of the seedlings was not affected significantly by 5×10^{-5} M of catechol and gallic acid, however, pyrogallic acid caused a significant decrease in these parameters at the same concentration (Table 3). The concentrations above 5×10^{-5} M decreased fresh and dry weight of the seedlings, irrespective of the phenolic compound used in the treatment. Out of the three phenolics used, pyrogallic acid was the inhibitoriest on the growth of seedlings.

The content of the total phenolics was found to be markedly higher in the seedlings treated with 5×10^{-5} M each of catechol, gallic acid and pyrogallic acid, compared to the control (Table 3). The increase was maximum with catechol and least with gallic acid. However, the concentrations above 5×10^{-5} M, brought about a proportionate decrease in the total phenol content in treated seedlings. The decrease in tissue phenols was the most prominent in seedlings raised from the seeds treated with gallic acid,

Table 1: Effect of Graded Concentrations (0, 5 × 10^{-5} M, 10^{-4} M, 5 × 10^{-4} M or 10^{-3} M) Each of Catechol, Gallic Acid and Pyrogallic Acid on the Percent Germination of Cucumber (*Cucumis sativus* L.) cv Long Green, at 24, 48 and 72 Hours of Sampling of Germination (NS = Non-significant)

Concentration (M)	Catechol			Gallic Acid			Pyrogallic acid		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
0	8.5	46.9	80.9	8.5	46.9	80.9	8.5	46.9	80.9
5×10^{-5}	0	45.8	82.4	0	42.3	69.8	0	50.4	84.8
10^{-4}	0	45.3	79.4	0	45.2	53.2	0	46.91	70.0
5×10^{-4}	0	35.4	42.17	0	35.2	39.0	0	35.3	53.2
10^{-3}	0	32.12	35.27	0	10.4	15.4	0	4.9	8.0
LSD at 5%	NS	4.7	5.2	NS	7.7	5.9	NS	7.3	12.7

Table 2: Effect Of Graded Concentrations (0, 5 × 10^{-5} M, 10^{-4} M, 5 × 10^{-4} M Or 10^{-3} M) Each of Catechol, Gallic Acid and Pyrogallic Acid on the Radicle Length (Mm), and Hypocotyl Length (Mm), of the Seedlings of Cucumber (*Cucumis sativus* L.) Cv Long Green, at 72 h of Germination

Concentration (M)	Radicle Length (mm)			Hypocotyl Length (mm)		
	Catechol	Gallic Acid	Pyrogallic Acid	Catechol	Gallic Acid	Pyrogallic Acid
0	5.2	5.2	5.2	2.0	2.0	2.0
5×10^{-5}	5.0	4.7	3.0	1.8	1.7	1.4
10^{-4}	3.1	3.0	2.1	0.9	1.0	1.0
5×10^{-4}	3.0	2.7	1.8	0.9	0.8	0.6
10^{-3}	1.5	1.0	0.6	0.5	0.3	0.2
LSD at 5%	0.43	0.52	0.59	0.03	0.05	0.03

Table 3: Effect of Graded Concentrations (0, 5 × 10^{-5} M, 10^{-4} M, 5 × 10^{-4} M or 10^{-3} M) Each of Catechol, Gallic Acid and Pyrogallic Acid on the Fresh Weight (Mg), Dry Weight (Mg) and Total Phenolic Content in the Seedlings of Cucumber (*Cucumis sativus* L.) Cv Long Green, at 72h of Germination

Concentration (M)	Fresh Weight (mg)			Dry Weight (mg)			Total Phenolic Content		
	Catechol	Gallic Acid	Pyrogallic Acid	Catechol	Gallic Acid	Pyrogallic Acid	Catechol	Gallic Acid	Pyrogallic Acid
0	104.92	104.92	104.92	24.41	24.41	24.41	148.5	148.5	148.5
5×10^{-5}	106.26	100.78	83.21	24.95	24.10	20.35	269.5	175.5	247.0
10^{-4}	88.79	70.20	70.21	20.01	18.54	17.80	252.5	163.0	135.5
5×10^{-4}	84.47	58.43	44.40	16.50	14.25	14.66	188.0	103.4	135.0
10^{-3}	49.46	34.31	23.19	11.44	10.63	8.52	171.5	80.5	119.5
LSD at 5%	13.33	11.40	10.47	3.6	3.2	4.8	14.10	11.90	8.48

where the seedlings receiving concentration above 10^{-4} M possessed the total phenol content less than that of the water treated control. In the seedlings treated with pyrogallic acid, the level of tissue phenols was also below the water treated control but the decrease was not as pronounced as with gallic acid.

Seed germination in *sensu stricto* is a cumulative consequences of many physical, physiological, biochemical, cellular and molecular events rendering the radicle able to emerge from seed. They are well versed with intracellular bodies of lipids, proteins, carbohydrates, organic phosphate and various other inorganic compounds, which facilitate the process of germination and the growth of the resulting seedlings. These compounds are consumed during the course of germination by involving various enzymes such as hydrolases, lipases, proteinases and phosphatases are released and/or synthesized *de novo* to facilitate the availability of simpler substances to the embryo, for its growth (Salisbury and Ross, 1991). However, in the present research, treating the seeds with graded concentrations of phenolic compounds viz. catechol, gallic acid or pyrogallic acid resulted in a sharp decline in germination and seedling growth. The inhibitory effect of these compounds on early growth is not surprising since phenolic acids are potent germination and growth inhibitors (Einhellig, 1996; and Mizutani, 1999). For example, phenolics from barley affect the growth of other plants (Liu and Lovett, 1993). Such inhibitory impact of phenolics has also been reported in mung bean (Batish *et al*., 2008). Baleroni *et al*. (2000) demonstrated that another phenolic compound, p-coumaric acid at 1mM severely affects the root growth and fresh weight of canola (*Brassica napus*).

Since there are intreguing evidences which suggest a promising role of phytohormones in the seed germination and subsequent seedling growth. Tmaszewski and Thimann (1966) reported that monophenols stimulated the decarboxylation of indole-3-acetic acid (IAA). Similarly, some other phenolics compounds such as *p-coumaric* acid, *p-hydroxybenzoic* acid, vanillic acid, syringic acid, and phloretic acid stimulate IAA oxidase (Frank, 1986). Reports on the effects of phenolic acids on other hormones (Ray *et al.,* 1980; Li *et al*., 1993) have found that some regulatory polyphenols may reduce growth by binding with gibberellic acid (GA). Li *et al.* (1993) reported interactions of *t-cinnamic* acid, ferulic acid, chlorogenic acid), *p-coumaric* acids (II), coumarin and ABA on seedling growth and seed germination of lettuce. These phenolic compounds along with ABA had additive inhibitory effects, both on seedling growth and seed germination. Therefore, at present, it is difficult to pinpoint whether these phenolic acids interfered with hormones or whether they directly inhibited seed germination.

CONCLUSION

The phenolic compounds had an inhibitory impact on the seed germination and seedling growth in cucumber (*Cucumis sativus* L.). However, the inhibition was dose dependent.

REFERENCE

1. Baleroni C R S, Ferrarese M L L, Souza N E and Ferrarese-Filho O (2000), “Lipid Accumulation During Canola Seed Germination in Response to Cinnamic Acid Derivatives”, *Biol. Plant*., Vol. 43, pp. 313-316.

2. Batish D R, Singh H P, Kaur S, Kohli R K and Yadav S S (2008), “Caffeic Acid Affects Early Growth, and Morphogenetic Response of Hypocotyl Cuttings of Mung Bean (Phaseolus aureus)”, *J. Plant Physiol.,* Vol. 165, pp. 297-305.

3. Cheynier V (2005), “Polyphenols in Foods are More Complex Than Often Thought”, *Am. J. Clin. Nutr.*, Vol. 81 (suppl.), pp. 223-229.

4. Curir P, Van Sumere C F, Termini A, Barthe P, Marchesini A and Dolci M (1990), “Flavonoid Accumulation is Correlated with Adventitious Roots Formation in *Eucalyptus gunnii* Hook Micropropagated Through Axillary Bud Stimulation”, *Plant Physiol.,* Vol. 92, pp. 1148-1153.

5. Dakora F D, and Phillips D A (2002), “Root Exudates as Mediators of Mineral Acquisition in Low-nutrient Environments”, *Plant Soil*, Vol. 245, pp. 35-47.

6. Deladonde M, Barret Y and Coumans M P (1996), “Development of Phenolic Compounds in Maize Anthers (*Zea mays*) During

Cold Pre-treatment Prior to Endogenesis" *J. Plant Physiol.*, Vol. 149, pp. 612-616.

7. Einhellig F A (1996), "Interactions Involving Allelopathy in Cropping Systems", *Agron J.,* Vol. 88 pp. 886-893.
8. Frank A E (1996), "Mechanisms and Modes of Action of Allelochemicals", in Putnum A R and Tang C S (Eds.), *The Science of Allelopathy*, Whiley-Interscience Publication, pp. 171-188.
9. Hayat S, Ali B and Ahmad A (2007), "Salicylic Acid: Biosynthesis, Metabolism and Physiological Role in Plants", in Hayat S and Ahmad S (Eds.) *Salicyclic Acid: A Plant Hormone*, Springer, The Netherlands, pp. 1-14.
10. Kevers, Coumans M, Coumans G M and Gasper T (1984) "Physiological and Biochemical Events Leading to Vitrification of Plants Cultured *In Vitro*", *Physiol. Plant.*, Vol. 61, pp. 69-74.
11. Lawson C G R, Rolfe B G and Djordjevic M A (1996), "*Rhizobium* Inoculation Induces Condition Dependent Changes in the Flavonoid Composition of Root Exudates from *Trifolium subterraneum*", *Aust. J. Plant Physiol.*, Vol. 23, pp. 93-101.
12. Li H H, Inoue M, Nishimura H, Hasegawa K, Mizutani J and Tsuzuki E (I 993), "Interactions of *Trans-cinnamic* Acid, Its Related Phenolic Allelochemicals, and Abscisic Acid in Seedling Growth and Seed Germination of Lettuce", *J. Chern. Ecol.,* Vol. 19, pp. 1775-1787.
13. Liu D L, and Lovett J V. (1993), "Biologically Active Secondary Metabolites of Barley II, Phytotoxicity of Barley Allelochemicals", *J. Chem. Ecol.,* Vol. 19, pp. 2231-2244.
14. Manach C, Scalbert A, Morand C, Rémésy C and Jimenez L (2004), "Polyphenols: Food Sources and Bioavailability" *Am. J. Clin. Nutr.*, Vol. 79, pp. 727-747.
15. Mizutani J (1999), "Selected Allelochemicals", *Crit. Rev. Plant Sci.*, Vol. 18, pp. 653-671.
16. Ndakidemi P A and Dakora F D (2003), "Legume Seed Flavonoids and Nitrogenous Metabolites as Signals and Protectants in Early Seedling Development: Review", *Functional Plant Biol.*, Vol. 30, pp. 729-745.
17. Putnam A R and Tang C S (1986), *The Science of Allelopathy*, John Wiley and Sons, New York.
18. Ray S D, Gurupurasad K N and Laloraya M M (1980), "Antagonistic Action of Phenolic Compounds on Abscisic acid, Induced Inhibition of Hypocotyl Growth", *J. Exp. Bot.,* Vol. 31, pp.1651-1656.
19. Salisbury F B, and Ross C W (1991), *Plant Physiology*, Wadsworth Publishing Company, California, USA.
20. Scalbert A, Johnson I T, Saltmarsh M (2005), "Polyphenols: Antioxidants and Beyond", *Am. J. Clin. Nutr.*, Vol. 81 (suppl), pp. 215-217.
21. Takahama U and Oniki T (1992), "Regulation of Peroxidase Dependent Oxidation of Phenols in the Apoplast of Spinach Leaves by Ascorbate", *Plant Cell Physiol.*, Vol. 33, pp. 379-387.
22. Tmaszewski M and Thimann K V (1966), "Interactions of Phenolic Acids, Metabolic Ions and Chelating Agents on Auxin-Induced Growth", *Plant Physiol.*, Vol. 41, pp. 1433-1454.
23. Vidhyasekaran P (1988), *Physiology of Disease Resistance in Plants*, Vol. 1, CRC Press, Boca Raton, FL.