Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2022; 10(2): 13-19

Received: 07-01-2022
Accepted: 10-02-2022

Movva Vijaya
Applied Biology Division, CSIR-Indian Institute of Chemical Technology Tarnaka, Hyderabad, Telangana, India

Pathipati Usha Rani
Applied Biology Division, CSIR-Indian Institute of Chemical Technology Tarnaka, Hyderabad, Telangana, India

RS Prakasham
Organic Synthesis and Process Chemistry Division, CSIR-Indian Institute of Chemical Technology Tarnaka, Hyderabad, Telangana, India

Corresponding Author:
Pathipati Usha Rani
Applied Biology Division, CSIR-Indian Institute of Chemical Technology Tarnaka, Hyderabad, Telangana, India

Spodoptera litura F. induced cross-resistance against microbial pathogens in *Capsicum annuum* L.

Movva Vijaya, Pathipati Usha Rani and RS Prakasham

DOI: https://doi.org/10.22271/j.ento.2022.v10.i2a.8962

Abstract
Herbivory by *Spodoptera litura* (F) larvae stimulate the production of induced defense in chili plants as a defense response. The induced defense response of the plant causes a change in the secondary metabolites. The present study was conducted hypothesizing the role of pest induced defense in inhibiting the successive invading pathogens (Cross-resistance) in chili plants. Bioassay studies were conducted to test our hypothesis and observed herbivory significantly reduced invasion of the bacterial pathogen, *Xanthomonas campestris* pv. Vesicatoria. As the phenolic acids in the plants are known to possess antimicrobial properties, we presumed the role of *S. litura* induced phenols in inhibiting the bacterial growth in chili plants as well. An *in vitro* agar dilution assay was conducted to identify the effective phenolic compound with antimicrobial nature by evaluating the minimum inhibitory concentrations. Overall, syringic acid was found to be effective in inhibiting *X. campestris* pv. vesicatoria growth, as indicated by low minimum inhibitory concentrations (MICs). The results indicate the cross-resistance role of herbivory induced secondary metabolites.

Keywords: *Xanthomonas campestris* pv. vesicatoria, *Colletorichum capsici*, *Capsicum annuum*, *Spodoptera litura*, phenolic acids, metal accumulation

Introduction
Plants experience a range of biochemical and antioxidative enzymatic changes due to the biotic stress caused by the phytophagous insects as a defensive response. Plant defenses are constrained by nutrient variation in the environment and hypothesized the synthesis of secondary metabolites is in ordinance with C: N ratio according to the carbon: nutrient balance hypothesis [1]. This hypothesis predicts that changes in available nutrients will change the palette of defenses. Plant defense and growth are both fuelled by compounds synthesized from a shared pool of carbon and nitrogen, implying the existence of competition for carbon and nitrogen allocation to both metabolisms. Metal elements, commonly present in the plant, aid in growth and development. In addition to their effect on determining the growth, this metal element poses beneficial effects on interactions of plants with biotic [2] and abiotic stress factors. Herbivory also induces the production of secondary metabolites in plants which may play a role in direct defense against the feeding pest [3]. The augmented accumulation of phenols and flavonoids under herbivory in many plant systems is proved to be involved in host plant defense against pathogens [4]. Sometimes, herbivory induces cross-resistance in plants to prevent or reduce the invasion of succeeding pathogens. This phenomenon of cross-resistance in plants under herbivory is evidenced in many plant systems such as soybean [5], bitter dock [6], tomato [7, 8]. The occurrence of induced phenolics in plants is presumed to play a role in inhibiting the biotrophic pathogen invasion into the infested plants. An exceptional example is the resistance of phenolic stilbenes to fungal colonization in grape plants [9]. The antimicrobial properties of phenylpropanoid pathway intermediates derivatives have been well demonstrated in in-vitro experiments [10, 11]. Also, a fungal pathogen *Pyricularia oryzae*, in rice, has confirmed growth inhibition upon treatment with the naringenin, kaempferol, quercetin and dihydroquercetin [12]. Likewise, spraying *Arabidopsis thaliana* plants with quercetin was identified as a prime defense response against *Pseudomonas syringae* pv. Tomato DC300 (Pst) [13].

In this study, we aimed to understand how plants integrate insect-induced signals into specific defense responses against plant pathogens. Because of the nature of the response of *Capsicum annuum* to feeding by *S. litura* [14], we hypothesized that pest larval feeding induced chemicals might prompt resistance in chili plants by being effective towards the major plant pathogens of chili.

Hence, we studied the ability of insect feeding induced plant phenols and their toxic properties against the major bacterial (*Xanthomonas campestris* pv. vesicatoria) and fungal pathogens (*Colletotrichum capsici*) of the *C. annuum* plants, and the study was designed to understand the role of *S. litura* induced phenol production in inhibiting the growth of pathogens that subsequently infest the host plants. Additionally, we also analyzed the effect of herbivory on plant's metal and elemental compositions to correlate their role in cross-resistance mechanisms.

Xanthomonas campestris pv. vesicatoria is a gram-negative bacterium which causes bacterial leaf spots in chili. The leaves exhibit small, circular or irregular, dark brown or black greasy lesions and are usually smaller than 3 mm in diameter depending on the hot and humid conditions. Affected seedlings develop yellow spotting and mat defoliate when the infection is severe. *Colletotrichum capsici* is one of the significant plant pathogens causing 'anthracnose' in economically important crops like legumes, vegetables, cereals and perennial crops [15]. Among these, chili (*Capsicum* spp.), an important economic crop worldwide, is severely affected by anthracnose, which leads to a considerable yield loss [16]. Characteristic symptoms of anthracnose include sunken necrotic tissues, with concentric rings of acervuli on chili fruits.

Materials and Methods

Pest and Plant Maintenance

The initial culture of *Spodoptera litura* F (NBAII-MP-NOC-02) pupae was procured from ICAR- National Bureau of Agricultural Insect Resources (NBAIR), Bangalore. Neonate larvae of *S. litura* were kept in plastic tubs (25-cm diameter) and were provided with fresh chili leaves, under controlled room conditions of 28 ± 2 ºC, 65 ± 5% relative humidity and a photoperiod of 16:8 (Light: Dark). Healthy third instar larvae of *S. litura* were used for the experiments. *Capsicum annuum* L. seedlings of the 'Garima' variety were obtained from the nursery of Acharya N. G. Ranga Agricultural University (ANGRAU), Rajendranagar, Hyderabad, Telangana, India. The seedlings were then transferred into earthen clay pots filled with a mixture of soil and vermicompost (2:1). Plants were maintained free of any pests and no chemical/ botanical pesticides were used. Uniform greenhouse conditions (30±5ºC, 60±5% relative humidity and 16: 8 Light: Dark photoperiod) were maintained and care was taken to avoid any kind of mechanical damage throughout their growth.

Pest feeding

Pre-starved (for about 4 h) third instar *S. litura* larvae (five to six numbers) were released on the chili plants and allowed to feed for 2 h. the insects were separated from the plant after 2 h. the pest fed leaves were then excised to their petioles after 24h after damage and were considered as insect fed leaves in all the experiments (quantification of metals, carbon and nitrogen content and identification of phenolics).

Quantification of Metal Content

The metal accumulation in the plant as a response to insect feeding was estimated using atomic absorption spectroscopy (AAS). The samples that were collected from the pest fed and unfed chili plants were dried and powdered and 0.2 gm of this sample was taken and processed according to the method suggested by [2]. Briefly, the leaf sample was taken into a Kjeldahl flask and mixed with sulfuric acid, perchloric acid, and nitric acid at the ratio of 1:4:40 by volume, respectively and left overnight to avoid foaming. The samples were initially boiled at 70 °C on a hot plate and were further heated up to 120 °C. Complete digestion of the leaf material was ensured by the formation of white fumes. The digests were then diluted with 10 ml of distilled water and heated for another 15 min. Further, they were cooled and transferred to volumetric flasks diluted with distilled water up to 50 ml. The resulted solution was filtered, and the samples were utilized for the elemental analysis using AAS (AAnalyst-Perkin-Elmer, USA) with hollow cathode lamps. Different elements in the sample were determined by comparing them with the standard calibration curves of various elements [17].

Quantification of Carbon and Nitrogen Content

For this, leaf samples were rinsed twice with deionized water to remove dust and soil, oven-dried at 60°C for 72 h, and then finely ground for measurement of C and N concentrations. Carbon and nitrogen concentration in leaves were measured with a CHNS/O Elemental Analyzer (Perkin-Elmer, USA).

Identifying pest induced phenols through HPLC

Phenolics Extraction

The plant sample extraction was done according to the method of [3] Movva and Pathipathi (2017) with slight modifications. Briefly, dried leaf material for 24 h; 2.5 g of dried leaf material (at 50 °C for 24 h) from pest fed and unfed plants were placed separately in 50 ml water in a rotating shaker for 24 h at 200 rpm. Water extracts were vacuum filtered (Whatman no. 2) followed by centrifugation at 12,500 g rpm for 20 min at 8°C, and then filtered through a 0.2 µm membrane for total phenol analysis. pH of the water extracts was lowered to 2.6–3.0 with distilled H_3PO_4. Extracts were partitioned with an equal volume of diethyl ether in aliquots of 5 ml twice by shaking the mixture approximately for 1 min and allowed to stand until diethyl ether partitioning occurred. The pooled partitioned volumes of diethyl ether were dispensed into a 50-ml Erlenmeyer flask and evaporated to near dryness in a suction chamber. Residues in the flask were dissolved in 2 ml methanol and filtered using 0.2 µm strite membranes. The filtered sample was injected into the HPLC column.

HPLC Analysis

The phenolic acid content in herbivore infested chili plants was analyzed using High-Performance Liquid Chromatography (HPLC) according to the method described by [3]. Briefly, the separation of phenolic compounds was accomplished on a Gilson (GX-271) semi-preparative HPLC system with a C_{18} column (2.5 × 30 cm^2 Gilson apparatus) and a liquid handler with an auto-injector was employed. A gradient elution programme was applied and elution was done with solvent A [acetic acid: water (2:98 v/v)] and solvent B [acetic acid: acetonitrile: water (2:30:68 v/v)] as a mobile phase for analysis of phenolic acid. The initial condition was programmed as 100% A; 0–5 min, changed to 100% B; 25–35 min, with a flow rate of 1.0 ml min^{-1} and the sample injection volume was 100µl. The signals were detected at 280 nm. Retention times for the standard compounds and the major peaks in the extract were recorded. Identification and determination of the separated compounds were made by comparison of retention time with that of standard compounds (Sigma Chemical Co., St. Louis, MO). All the experiments

were performed in five independent replicates.

Bacterial cultures

Xanthomonas campestris pv. vesicatoria was originally isolated from *Capsicum* sp. Bacteria were grown on a nutrient agar medium with glucose at 28 ±2ºC for 48h. The bacterial cells were suspended in sterile distilled water and centrifuged at 3000rpm. The pellets were re-suspended in distilled water and adjusted to the density of 10^8 CFU/ml (for plant inoculation).

Fungal cultures

Colletotrichum capsici (MTCC 10147) was obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The Fungal pathogen was maintained on potato dextrose agar (PDA, pH-7.0) slants at 28-32º C for 7-10 days (until the formation of colonies). The spore suspension was prepared minutes before each inoculation at a concentration of 1 x 10^6 conidia/ml, adjusted by counting in a Neubauer chamber (for plant inoculation).

Plant bioassays

For plant bioassays, 150 chili plants were selected and were divided into three groups of 50 each and were tagged as Plant group- A (PG-A), Plant group- B (PG-B), and Plant group- C (PG-C). For PG-A, plants were just sprayed with the similar volume of the microbial suspensions. For PG-B, the plants were mechanically damaged; the expanded leaves were damaged by scratching the leaf with a punch, accounting for 25% of the total leaf area. Six to ten third instar *S. litura* larvae were allowed to feed on PG-C chili plants for 3 h.

Each group was then divided into two parts of 25 plants and were used for growth inhibition assays with the bacterial pathogen, *X. campestris* pv. vesicatoria and the fungal pathogen, *C. capsici* as described by [36]. Challenge inoculations were performed after 24 h of the caterpillar damage and incision. The plants that were inoculated with the bacterial pathogen, *X. campestris* pv. vesicatoria were observed for disease symptoms after 7-10 days of inoculation, the percentage of leaves with disease symptoms (as characterized by [19]) was determined per plant. The plants that were inoculated with the fungal pathogen, *C. capsici* were observed for disease symptoms at 10-12 days after challenge inoculation, and disease severity (symptoms as characterized by [20] was determined. The experiments were repeated 5 times.

***In vitro* studies using Induced phenolic acids**

Vanillic acid, sinapic acid, chlorogenic acid, rutin, and syringic acid were purchased from Sigma (St. Louis MO). The above phenolic compounds were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions respectively and screened by using the good diffusion method [21]. To determine whether DMSO has any effects on *X. campestris* pv. vesicatoria and *C. capsici* growth inhibition, control experiments were carried out using culture media supplemented with DMSO. The minimum inhibitory concentration (MIC) for each phenolic compound was determined after respective incubation periods of bacteria and fungi.

Statistical analysis

Bioassay results from individual experiments were analyzed using one-way ANOVA. The statistical analysis was performed using Graph Pad Prism 5.0 (Graph Prism, Inc., San Diego, CA).

Results

Meta accumulation upon herbivory

A rapid decrease in Mg levels in the *S. litura* fed plants was observed compared to their levels in uninfested plants. Iron and Zn content increased in the chili plants upon *S. litura* herbivory. (Table 1).

Table 1: Representation of metal concentration of control and *S. litura* infested chili plants.

Sample	Uninfested Plan	*S. litura* fed plant
Mg (mg/gm)	69.84±0.03	58.72±0.2a
Fe (mg/gm)	6.73±0.11	7.57±0.04a
Ca (mg/gm)	162.5±0.01	109.9±0.001a
K (mg/gm)	226.9±0.03	206.2±0.03
Zn (mg/gm)	0.51±0.01	0.86±0.02a

Values (mean ± SE; n = 20) followed by different letters in a column are statistically different at $P \leq 0.001$

Impact of herbivory on plant C and N content

Foliar C and N content were affected in the infested plants due to defoliation by *S. litura*. A decrease in the total N content in *S. litura* infested plants was recorded compared to uninfested plants (Table 2). Third instar herbivory caused a ($P \leq 0.001$) raise in the total C content to 41.41% Hence, *S. litura* larval feeding caused a rapid increase in the carbon deposition and a considerable decrease in the nitrogen deposition.

Table 2: Elemental composition (Carbon and Nitrogen content) in the uninfested and pest infested chili plants.

Sample	Carbon%	Nitrogen %
Uninfested plant	41.47±2.24	6.10±1.7
S. litura fed plants	16.36±1.7a	2.43±0.6a

Values are (mean ± SE; n = 20) followed by different letters are statistically different at $P \leq 0.001$

Accumulation of phenolic compounds upon S. litura feeding

Larval feeding on the chili plant leaves caused qualitative and quantitative changes in phenol production in the plant. The phenolic contents varied significantly in the *S. litura* fed plants compared to undamaged plants. Vanillic acid was observed only in the pest infested plants, while it is completely absent in the undamaged plants. The quantitative increase in the sinapic acid and syringic acid was recorded in the *S. litura* fed plants compared to undamaged plants. Interestingly, phenolic compounds such as chlorogenic acid and rutin contents decreased in the chili plants under herbivory when compared to undamaged plants. The observed quantities of the phenolic compounds are represented in Table 3 and their respective HPLC chromatograms were represented in Figure 1.

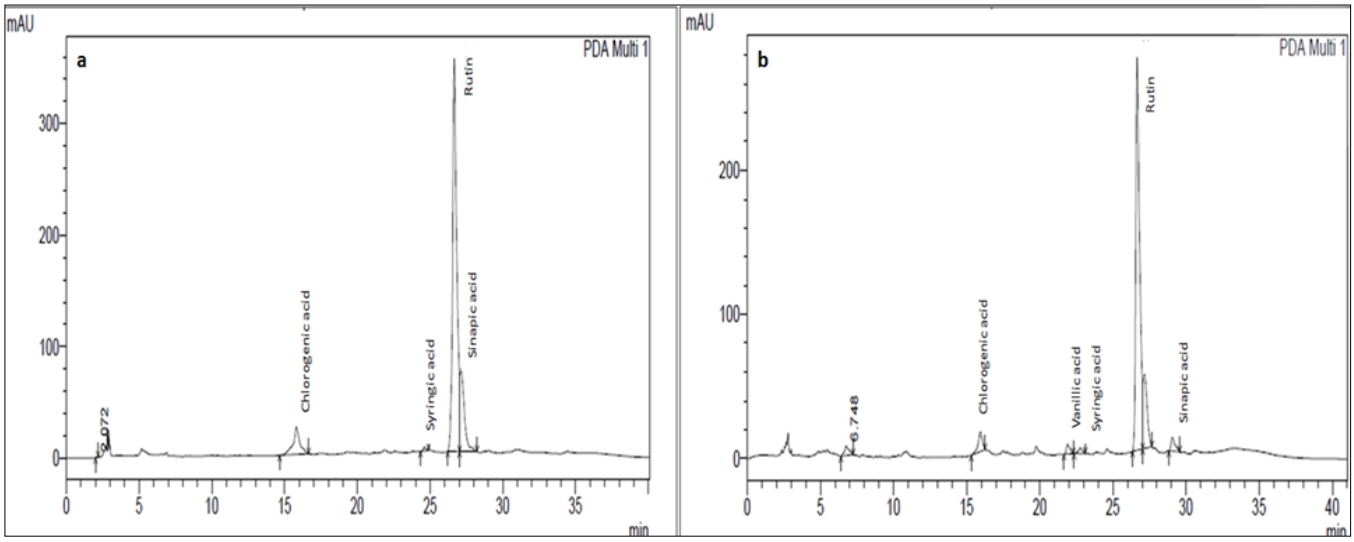

Fig. 1 HPLC chromatograms of a) Uninfested chili plants b) *S. litura* fed chili plants (infested)

Bioassays with X. campestris pv. vesicatoria and C. capsici

Bioassays with *X. campestris* pv. vesicatoria, a bacterial pathogen and *C. capsici*, a fungal pathogen were performed in the laboratory to understand the efficacy of pest induced chemicals against intruding pathogens. The chili plants that were induced with *S. litura* (PG-C) were recorded to oppose the bacterial attack. On the other hand, the *S. litura* fed plants inoculated with the fungal pathogen failed to inhibit the fungal colonization. In contrast, chili plants which were mechanically damaged (PG-B) and inoculated with the bacterial and fungal pathogens showed respective symptoms within 7 to 10 days post inoculation for *X. campestris* pv. vesicatoria and within 10-12 days post inoculation in case of *C. capsici*. Whereas, PG-A plants that were neither mechanically damaged nor herbivory fed showed less damage compared to PG-B (Figure 2 and 3). Inhibition of the bacterial growth on the *S. litura* infested chili leaves is assumed to be due to the presence of the induced plant phenols after herbivory.

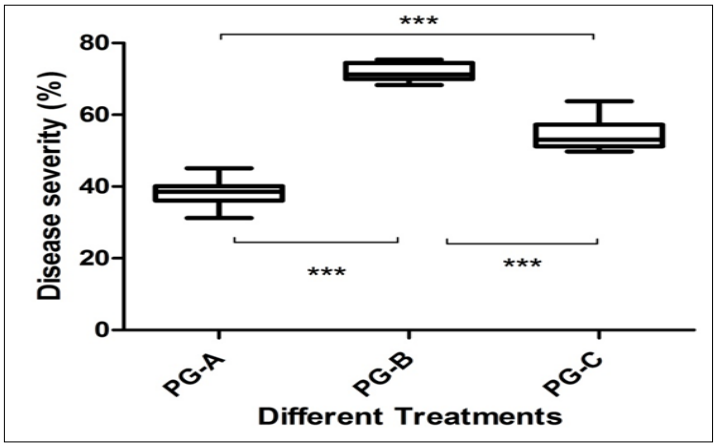

Fig 2: Effectiveness of herbivore-induced resistance against *X. campestris* pv. vesicatoria ((***- significant difference at P ≤ 0.001).

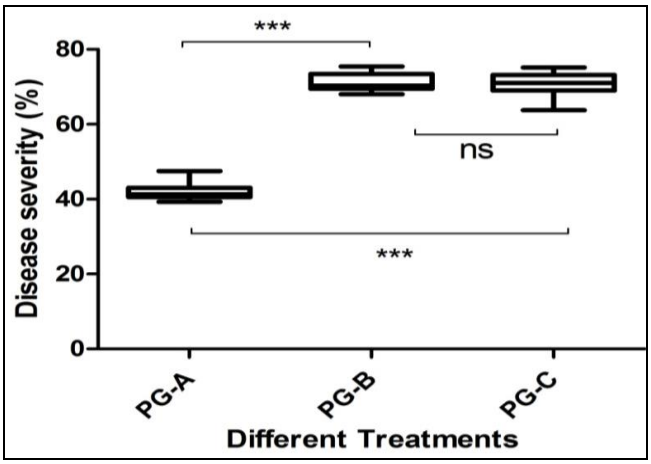

Fig 3: Effectiveness of herbivore-induced resistance against *C. capsici* (***- significant difference at P ≤ 0.001; ns=not significant).

Antibacterial and antifungal assay of pest induced plant phenols

Anti-bacterial and anti-fungal assays determined the efficacy of the induced phenolic compounds identified from infected chili plant leaves. The minimum inhibitory concentrations (MIC) for phenolic compounds were calculated after 7-10 days of incubation for the bacterial pathogen (*X. campestris* pv. vesicatoria) and 10-12 days for the fungal pathogen (*C. capsici*). Compatible results were observed in the repeated experiments with each compound. The MICs of phenolic compounds against *X. campestris* pv. vesicatoria are summarized in Table 4. The pest induced phenols failed to show any inhibition against the fungal pathogen *C. capsici* and hence the data was not included.

Table 3: Quantities of identified phenolic acids in chili leaves following herbivory by *S. litura*

Stage of the insect	Pest Unfed plants	*S. litura* fed plants
Vanillic acid	NI	2.86±0.03a
Sinapic acid	23.6±0.3	37.03±0.1b
Chlorogenic acid	8.1±0.04	6.85±0.01c
Rutin	75.3±0.3	56.8±0.1d
Syringic acid	0.313±0.01	0.565±0.02

The values indicate they mean phenolic acid as µg/g wet leaf, n = 5. Values followed by the letters are statistically significant at P ≤ 0.001.

Table 4: Minimum inhibitory concentration (MIC) of phenolic acids against *Xanthomonas campestris* (pv) vesicatoria

Bacterial strain	*X. campestris* (pv) vesicatoria
Vanillic acid (µM)	185.85±1.41
Sinapic acid (µM)	278.75± 2.03
Chlorogenic acid (µM)	352.79±2.18
Rutin (µM)	94.04±3.16
Syringic acid (µM)	78.84±1.95

Structure-Activity Relationship of Anti-Xanthomonas and Phenolic Compounds

Phenolic acid with two methoxy (–OCH3) substitutions (syringic acid) is more effective in inhibiting *X. campestris* pv. vesicatoria growth (Table 4) followed by the phenol with one methoxy substitution i.e., vanillic acid. Even though sinapic acid also possesses two methoxy groups its efficacy in inhibiting the bacterial growth was less compared to syringic acid. The mono-hydroxyl phenolic acid, syringic acid recorded the highest anti *X. campestris* pv vesicatoria activity than the phenols with di- and tri- hydroxyl groups of the compounds (Table 3). Overathe ll, hydroxyl (-OH) group at the para- position of the benzene ring appears to enhance the antibacterial (*X. campestris* pv. vesicatoria) activity of the tested phenolic compounds (Table 4).

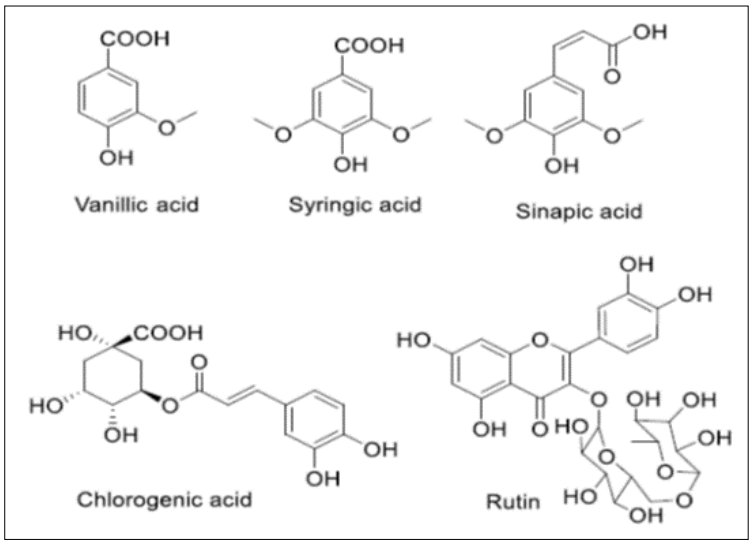

Fig 4: Chemical structures of phenolic compounds that are evaluated in the present study

Discussion

The changes in the metal concentrations in the infested plants indicate the defense response in the chili plants upon *S. litura* feeding. It is also evident from many studies that the accumulation of metals directly protects the plants from pathogen attack [22, 23]. The increased Zn content may play a role in inhibiting the bacterial growth on *S. litura* fed plants (PG - C) compared to that of PG-A and PG-B plants where they do not encounter the pest. The present result is supported by the earlier study, which stated that the accumulation of Zn in *Thlaspi caerulescens* (*Noccaea caerulescens*) inhibited the growth of *Pseudomonas syringae* [24]. This also confirms that the plants alter their defense in response to the stress. Accordingly, Zn depletion causes the nutrient deficiencies in the plant and as a result, it has been shown to increase susceptibility to a range of pathogens [25]. Therefore, it is clear that these accumulated metals in the infested plant assist in cross-resistance through conferring toxic effects against the invading pathogens.

Plants growing under natural conditions encounter simultaneous challenges from different external stresses so that different signaling pathways which can interact either synergistically or antagonistically enabling specific responses have evolved [26, 8]. Upon *S. litura* feeding, chili plants escalate a defense response by enhancing the phenolic acid production, which subsequently prevents the further infestation by the same herbivore [3]. This confirms the previous findings [27, 28, 29] in other plant species. Because of the dual role of herbivore-induced defense in both pathogen and insect resistance, we investigated whether herbivory by *S. litura* triggers cross-resistance against microbial pathogens. Our data shows that herbivore-induced resistance in chili is futile against the fungal pathogen *C. capsici*, but potent against the bacterial pathogen *X. campestris* pv. vesicatoria.

A recent study by [30] on plant phenolic compounds from essential oils confirmed the antimicrobial property of the plant

phenols. In the present study, we determined the effect of pest induced phenolic compounds on *X. campestris* pv. vesicatoria and *C. capsici* growth. Phenols are known to be very important in plant resistance against pathogenic bacteria and fungi. Among all the phenolic acids tested in the present study, syringic acid acted as a potent antibacterial agent against the bacteria, *X. campestris* pv. vesicatoria and showed no influence in inhibiting the fungi, *C. capsici*. Different results were recorded with syringic acid as fungi toxic against *Ganoderma boninense*, which causes Basal Stem Rot (BSR) in oil palm [31]. Rutin appears to be the following potent compound after syringic acid followed by vanillic acid against *X. campestris* pv. vesicatoria. The antibacterial activity of rutin was also reported on other bacterial species, such as *X. campestris*, *Agrobacterium tumefaciens*, *Xylella fastidiosa* etc [32, 33]. The possible mechanism of action as a potent antibacterial phenolic compound is presumed by [34, 35].

According to the results obtained in our study, we assume that herbivore-induced plant phenolic compounds are playing a major role in the resistance of plants to pathogen infestation. Syringic acid and rutin are the most promising of all the induced phenols in inhibiting the bacterial growth. We confirm the highest antibacterial efficacy of these compounds on the *X. campestris* pv. vescatoria. In the future, it may be possible that crops with improved resistance to *X. campestris* pv. vescatoria caused disease can potentially be produced by elevating the endogenous anti-Xanthomonas phenolic concentrations as an alternative to introducing antibacterial compounds.

Acknowledgments

Authors are thankful to the Director, CSIR-IICT, Hyderabad, India, for encouragement and analytical facilities provided to carry out this work.

References

1. Bryant JP, Chapin FS III, Klein DR. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos. 1983;40:357-368.
2. Pratyusha S, Usha Rani P. Induction of phenolic acids and metals in *Arachis hypogaea* L. plants due to feeding of three lepidopteran pests. Arthropod Plant Interactions. 2013;7:517-525.
3. Movva V, Pathipati UR. Feeding induced phenol production in *Capsicum annuum* L. influences *Spodoptera litura* F. larval growth and physiology. Archives of Insect Biochemistry and Physiology, 2017. https://doi.org/10.1002/arch.21387
4. Boudet AM. Evolution and current status of research in phenolic compounds. Phytochemistry. 2007;68:2722-2735.
5. Padgett GB, Russin JS, Snow JP, Boethel DJ, Berggren GT. Interactions among the soybean looper (Lepidoptera: Noctuidae), three-cornered alfalfa hopper (Homoptera: Membracidae), stem canker, and red crown rot in soybean. Journal of Entomological Science. 1994;29:110-119.
6. Hatcher PE, Paul ND. Beetle grazing reduces natural infection of *Rumex obtusifolius* by fungal pathogens. New Phytologist. 2000;146:325-333.
7. Stout MJ, Workman KV, Bostock RM, Duffey SS. Specificity of induced resistance in the tomato, Lycopersicon esculentum. Oecologia, 1998a; 113: 74-81.
8. Bostock RM, Karban R, Thaler JS, Weyman PD, Gilchrist D. Signal interactions in induced resistance to pathogens and insect herbivores. European Journal of Plant Pathology. 2001;107:103-111.
9. Jeandet P, Douillet-Breuil AC, Bessis R, Debord S, Sbaghi M, Adrian M. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. Journal of Agricultural and Food Chemistry. 2002;50:2731-2741.
10. Barber MS, McConnell VS, DeCaux BS. Antimicrobial intermediates of the general phenylpropanoid and lignin-specific pathways. Phytochemistry. 200;54:53-56.
11. Dixon RA. Natural products and plant disease resistance. Nature. 2001;411:843–847.
12. Padmavati M, Sakthivel N, Thara KV, Reddy AR. Differential sensitivity of rice pathogens to growth inhibition by flavonoids. Phytochemistry. 1997;46:499-502.
13. Jia ZH, Zou BH, Wang XM, Qiu JA, Ma H, Gou ZH, *et al*. Quercetin-induced H_2O_2 mediates the pathogen resistance against *Pseudomonas syringae* pv. tomato DC3000 in *Arabidopsis thaliana*. Biochemical and Biophysical Research Communications. 2010;396:522-527.
14. Vijaya M, Usha Rani P. Defensive responses in *Capsicum annuum* (L) plants, induced due to the feeding by different larval instars of *Spodoptera litura* (F). Arthropod Plant Interactions. 2017;11:193-202.
15. Bailey JA, Jeger MJ. *Colletotrichum*: Biology, Pathology and Control. Wallingford: Common wealth Mycological Institute, 1992, 388.
16. Pakdeevaraporn P, Wasee S, Taylor PWJ, Mongkolporn O. Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in Capsicum. Plant Breeding. 2005;124:206-208.
17. Zafar M, Khan MA, Ahmad M, Jan G, Sultana S, Ullah K, *et al*. Elemental analysis of some medicinal plants used in traditional medicine by atomic absorption spectrophotometer (AAS). Journal of Medicinal Plants Research. 2010;4:1987-1990.
18. Wakuma Biratu, Derbew Belew, Edossa Ettissa. Evaluation of hot pepper (*Capsicum annuum* L.) cultivars for growth and dry pod yields against different blended fertilizer and nitrogen rates in raya Azebo, Southern Tigray. Int. J Res. Agron. 2021;4(2):15-22.
19. Jones JB, Pernezny K. Bacterial spot. In: K Pernezny. PD Roberts, JF Murphy & NP Goldberg (eds.) Compendium of pepper diseases. American Phytopathological Society, St. Paul, MN, 2003, 6-7.
20. Lewis-Ivey ML, Nava-Diaz C, Miller SA. Identification and management of *Colletotrichum acutatum* on immature bell peppers. Plant Diseases. 2004;88:1198-1204.
21. Bauer AW, Kirby MM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966;45:493-496.
22. Boyd RS, Shaw JJ, Martens SN. Nickel hyperaccumulation defends *Streptanthus polygaloides* (Brassicaceae) against pathogens. American Journal of Botany. 1994;81:294–300.
23. Ghaderian YSM, Lyon AJE, Baker AJM. Seedling mortality of metal hyperaccumulator plants resulting from damping off by *Pythium* spp. New Phytologist. 2000;146:219–24.

24. Fones H, Calum ARD, Arantza Rico, Fang Fang Smith JAC, Preston GM. Metal hyperaccumulation armors plants against disease. PLoS Pathogens, 2010. https://doi.org/10.1371/journal.ppat.1001093
25. Dordas C. Role of nutrients in controlling plant diseases in sustainable agriculture: A review. Agronomy for Sustainable Development Springer Verlag /EDP Sciences/INRA. 2008;28:33-46.
26. Walling LL. The myriad plant responses to herbivores. Journal of Plant Growth Regulation, 2000; 19: 195-216.
27. Kessler A, Baldwin IT. Plant responses to insect herbivory: the emerging molecular analysis. Annual Reviews of Plant Biology. 2002;53:299-328.
28. Agrawal AA, Kurashige NS. A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. Journal of Chemical Ecology. 2003;29:1403-1415.
29. Howe GA. Jasmonates as signals in the wound response. Journal of Plant Growth Regulation. 2005;23:223-237.
30. Zabka M, Pavela R. Antifungal efficacy of some natural phenolic compounds against significant pathogenic and toxinogenic filamentous fungi. Chemosphere. 2013;93:1051-6.
31. Chong KP, Atong M, Rossall S. The role of syringic acid in the interaction between oil palm and *Ganoderma boninense*, the causal agent of basal stem rot. Plant Pathology. 2003;61:953-963.
32. Taguri T, Tanaka T, Kouno I. Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. Biological and Pharmaceutical Bulletin. 2006;29:2226-2235.
33. Maddox CE, Laur LM, Tian L. Antibacterial activity of phenolic compounds against the phytopathogen *Xylella fastidiosa*. Current Microbiology. 2010;60:53-58.
34. Murakami S, Muramatsu M, Tomisawa K. Inhibition of gastric H+, K+-ATPase by flavonoids: a structure-activity study. Journal of Enzyme Inhibition and Medicinal Chemistry. 1999;14:151-66.
35. Guo R, Wei P, Liu W. Combined antioxidant effects of rutin and Vitamin C in Triton X-100 micelles. Journal Pharmaceutical and Biomedical Analysis. 2007;43:1580-1586.
36. Ton J, Van Pelt JA, Van Loon LC, Pieterse CMJ. Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in Arabidopsis. Molecular Plant Microbe Interactions. 2002;15:27-34.