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Biotic Stress Induced by Bacterocera cucurbitae (Melon Fly) Triggers Defense Related Phenylpropanoid Pathway (PPP) and ROS Detoxifying Enzymes in Cucurbits as Adaptation

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ABSTRACT

The enzymes of phenylpropanoid (PP) pathway and antioxidant systems are among the plants broad spectrum of defense strategies and biochemical changes that respond to biotic stress. Melon fly (Bacterocera cucurbitae) is one of the detrimental pests damaging the cucurbitaceous family. Present study attempted to investigate the various enzymatic changes and antioxidant potential to post infection in cucumber (Cucumis sativus) and Chayote (Sechium edule). The enzymes phenylalanine lyase (PAL), tryrosine alanine lyase (TAL) and cinnamyl alcohol dehydrogenase (CAD) of PP pathway were constitutively expressed in the fruit tissues. These were low in healthy (8%), apparent healthy (13%) and remarkably high in infected tissues (25%). The enhanced PP enzymes upon Bacterocera cucurbitae infection were further confirmed by the increased total flavanoids (0.075%) and phenolic (12%) in the infected part. Antioxidant potential as evaluated measuring the ROS detoxifying enzyme activity revealed that antioxidant activity was increased in apparent healthy (50%) and infected tissue (60%) compared to the healthy tissue (37%). Both PP pathway and antioxidant enzymes were found significantly higher in infected chayote tissues as compared to cucumber and duration required for infection establishment in chayote (10 days) is also longer than cucumber (6 days). Considering these observations study indicates B. cucurbitae infestation in these fruits lead to up regulation and accumulation of PP pathway enzymes resulting in increased lignin synthesis which provide resistance for establishment of infection. The increased antioxidant potential combat and adapt to the infection induced biotic stress. The observed response in the chayote is markedly higher in upregulation of enzymes than in the cucumber which could be reason for its reported resistance against B. cucurbitae and susceptibility of cucumber. The melon fly is a damaged to many fruits and vegetable crops, especially 20 to 50% cucurbits fruits, fruits protect them self against melon fly damaged by used of various defense mechanism. Fruits inducible defense mechanism response with secondary metabolites and protein that act as directly and indirectly to insect caused avoid.

Keywords: Phenylpropanoid pathway (PPP) enzymes, Reactive oxygen species, *Bacterocera cucurbitae*, Cucumber (*Cucumis sativus*), Biotic stress, Chayote (*Sechium edule*)

Abbreviations: APH: Apparent Healthy Tissue; CAD: Cinnamyl Alcohol Dehydrogenase; CAT: Catalase; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; EDTA: Ethylene Di Amine Tetra Acetic Acid; H_2O_2: Hydrogen Peroxide; HT: Healthy Tissue; INF: Infestation Tissue; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; NBT: Nitrogen Blue Tetrazolium; O_2: Superoxide; PAL: Phenylalanine Lyase; PMS: Phenylmethane Sulphonyl Fluoride; PPP: Phenylpropanoid Pathway; ROS: Reacting Oxygen Species; SOD: Superoxide Dismutase; TAL: Ttryrosine Alanine Lyase; TBA: Thiobarbutaric Acid

INTRODUCTION

Plants are constantly exposed to diverse number of biotic and abiotic stresses. As a stress response, plant defense mechanisms display is coordinated and integrated set of metabolic alterations in an attempt to adapt to stress [1]. In response to microbial attack, plants activate defense responses that lead to induction of a broad spectrum antibacterial and antifungal [2] compounds some of which may be species specific. These induced defense mechanisms are expressed in the site of damaged (hypersensitive response) as well as at a distance (signaled by methyl salicylate) to the site of primary infestation and protect the plant from the spread of the infection and future attack [3]. Phenylpropanoids (PPs) belongs to the largest group of secondary plant metabolites (including lignin, flavanoids, phytoalexins, tannins, etc.) produced from plants in response to biotic and abiotic stresses and Phenylpropanoid (PP) compounds in plant defense mechanism from preformed or reduced physical and chemical barriers against infection. Phenylalanine ammonia-lyase (PAL) enzymes the first step in the biosynthesis of phenylpropanoids pathway, which form a wide variety of plant secondary products which includes flavonoid pigments, lignin, antimicrobial phenolic, UV protectants and cell wall associated phenolic that are synthesized in response to normal developmental causes, pathogen attack, and mechanical and UV stress [4]. Induction of phenolic compounds biosynthesis is a classic defense reaction to pathogens attack [5]. Phenolic acids are the main polyphenols made by plants which has an important role in plant-microbe interactions and symbiosis. The formation of cinnamic acid from phenylalanine mediated by PAL is a pivotal branch point of primary and secondary metabolism important regulatory step in the formation of many phenolic acids [3].

The oxidative burst is one of the earliest and more defense mechanism reactions in plants by pathogen invasion and many other stresses. Reactive oxygen species (ROS) produced as a result of response to stress becomes vital for plant defense during plant-pathogen interactions [6]. Rapid generation of ROS in the apoplast, as superoxide (O_2) and hydrogen peroxide (H_2O_2) seems to play a central role in this oxidative burst, acting as a signal for localized death of challenged cells (hypersensitive reaction) and as a diffusible signal for the induction of cellular protecting genes in adjacent cells [7]. But despite this, accumulating evidences have showed that there is a state of balance maintained between ROS generation and scavenging, as increased ROS level is reported to be detrimental for the cell viability [8]. Therefore, the production of high levels of ROS also concomitantly stimulated the synthesis of antioxidant and detoxifying compounds such as SOD, peroxidases and other antioxidant like phenolic compounds.

Bacterocera cucubitae (Melon fruit fly) belonging to diptera is one of the world widely distributed detrimental plant pest that damages about 81 host plants mainly from the cucurbitaceae family [9]. The melon fly around 700 species widely distributed in temperate, sub-tropical and tropical and detrimental pest that damaged about 81 host plants [10]. The cucumber is highly susceptible for melon fly infestation however; chayote which belongs to same family is highly resistant to melon fly infestation. The present study focus to investigate the possibility of biochemical modulation and regulation of the phenylpropanoid pathway (PPP) enzymes (PAL, TAL and CAD) and antioxidant enzymes (SOD, catalase) upon the infestation of melon fly in cucumber. This study will provide the comparative analysis of the defense responses involving PPP and antioxidant enzymes in cucumber and chayote on post *B. cucurbitae* infestation. The evaluation will provide an insight into the defensive mechanism and regulatory pathways to understand resistance of chayote over susceptibility of cucumber.

MATERIALS AND METHODS

Chemical and reagents

Ammonium molybdate, nitrogen blue tetrazolium (NBT), riboflavin, 2-deoxyribose, EDTA, thiobarbutaric acid (TBA), ferric chloride, ascorbic acid, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), guaiacol, NADPH, *t*-cinnamic acid, phenylmethane sulphonyl fluoride, *p*-coumaric acid, L-phenylalanine and L-tyrosine were procured from HiMedia Laborotories, India. All other chemicals and reagents used in this experiment were of analytical grade. *Bacterocero cucurbitae* pupae (Melon fly) were obtained from the Department of Entomology, Indian Institute of Horticulture Research (IIHR), Bengaluru.

Melon fly culture

The melon flies were cultured in according to the methods described previously with slight modifications [9]. Briefly, the insects were maintained on a laboratory diet comprising of yeast, casein and sugar in the ratio 1:1:2. Water provisioned as wet cotton was placed in Petri plates separately. Melon flies were given a 6 h access to 5 day old cucumber fruits for oviposition. Oviposited cucumbers were placed on a thin layer of moist sand in small plastic trays. Pupae were collected and kept in separate cages for adult emergence.

Plant material and extraction of tissue samples

Cucumber and chayote were obtained from the local agriculture field in Shimoga District, Karnataka. The tender cucumber and chayote were provisioned for melon fly oviposition in cage for 3-4 days. The infected plant material was then used for further investigations. The melon fly infested cucumber and chayote were subjected to sampling of tissues as described previously [11]. The tissues were graded into infected, apparent healthy and healthy group. The brown spot at the site of infestation tissue (INF) by insect was considered 'affected'. The tissue immediately surrounding the affected spot and remaining free of symptoms was treated as 'apparently healthy (APH)' (). Fruit remaining free of infestation was used for collection of 'healthy tissue' (HT) for comparison.

Oviposition and puncture studies in cucurbits fruits

Melon fly studies in resistance chayote and susceptible cucumber in various stages depends on the determination for melon fly damaged in oviposition (Tables 1 and 2). The damaged region are measured stage by stage in chayote Stage I- 8 cm × 1.6, Stage II- 8 cm × 2.8 cm, Stage III- 8 cm × 3.8 cm, Stage IV- 8 cm × 5.6 cm Stage V- 8 cm × 7.8 cm and in Cucumber Stage I- 12 cm × 5 cm, Stage II- 12 cm × 7 cm, Stage III- 12 cm × 10 cm, Stage IV- 18 cm × 12, and Stage V- 20 cm × 14 (Figures 2-4).

***In vitro* assay for phenylpropanoid pathway enzymes**

The enzymes Phenylammonia lyase (PAL) and Tyrosine ammonia lyase (TAL) of phenylpropanoid pathway were extracted and estimated by the previously described methods [12,13]. Briefly, one gram of sample was ground in 10 mL of 50 mM sodium borate buffer (pH 8.0 and 8.5) for PAL and TAL, respectively. The homogenate was centrifuged at 5000 g for 15min at 4°C. The supernatants were used for assays of enzyme activities.

Phenylalanine ammonia-lyase (PAL) assay

Phenyalanine ammonia-lyase was assyed using the reaction mixture consisting of 0.5 mL of enzyme extract and 150 mM of L phenylalanine and was adjusted to 3 mL with the extraction buffer. Incubation was done at 40 ± 1°C for 30 min. Phenylalanine ammonia-lyase activity was determined in terms of cinnamic acid at 290 nm. The concentrations of *t*-cinnamic acid were determined from the standard curves prepared by plotting absorbance at 290 nm. Specific

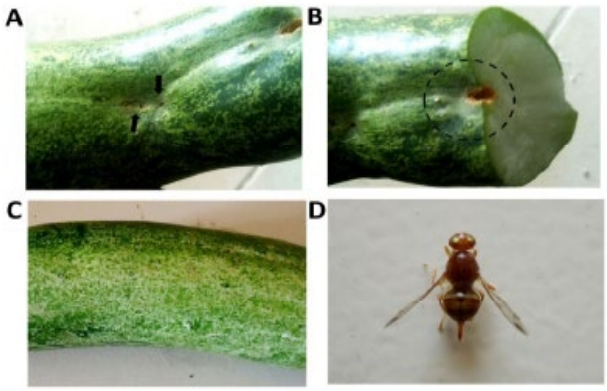

Figure 1: Healthy and melon fly (*B. cucurbitae*) infected cucumber and enlarged view of the in house cultured melon fly from egg. A. Melon fly infected cucumber. B. enlarged view of infection and infected tissue. C. Healthy non-infected cucumber. D. Melon fly-pest infecting the cucurbittae

Table 1: Effect of the cucurbits fruit Skin, Latex and Tissue inhibition with second instar larvae for melon fly

(Note: +++ More Active, ++ Moderactive, + Less active and --- Dead)

	Cucumber						Chayote					
Concentration of various cucurbits fruits extract	**Skin**		**Latex**		**Tissue**		**Skin**		**Latex**		**Tissue**	
Observation of larva Wt	1:1	1:5	1:1	1:5	1:1	1:5	1:1	1:5	1:1	1:5	1:1	1:5
0 to 5 h	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+++	+++
5 to 10 h	++	+++	++	++	+++	+++	++	++	+	++	+++	+++
10 to 20h	++	++	+	+	+++	+++	++	++	---	+	+++	+++
20 to 40 h	++	++	+	+	+++	+++	---	---	---	---	+++	+++
40 to 80 h	++	++	+	+	+++	+++	---	---	---	---	+++	+++

Table 2: Effect of the cucurbits fruit skin, latex and tissue in growth factor with second instar larvae for melon fly

	Cucumber						Chayote					
	Skin		Latex		Tissue		Skin		Latex		Tissue	
Observation of larva Wt	1:1	1:5	1:1	1:5	1:1	1:5	1:1	1:5	1:1	1:5	1:1	1:5
0 to 5 h	0.015 g	0.015 g	0.013 g	0.013 g	0.014 g	0.013 g	0.014 g	0.014 g	0.015 g	0.013 g	0.015 g	0.014 g
5 to 10 h	0.017 g	0.016 g	0.014 g	0.014 g	0.015 g	0.014 g	0.015 g	0.016 g	0.014 g	0.011 g	0.016 g	0.016 g
10 to 20h	0.018 g	0.017 g	0.016 g	0.015 g	0.016 g	0.016 g	0.014 g	0.015 g	0.013 g	0.010 g	0.017 g	0.016 g
20 to 40 h	0.018 g	0.017 g	0.016 g	0.015 g	0.017 g	0.016 g	0.013 g	0.014 g	0.011 g	0.010 g	0.017 g	0.017 g
40 to 80 h	0.020 g	0.018 g	0.017 g	0.016 g	0.019 g	0.017 g	0.013 g	0.013 g	0.011 g	0.009 g	0.017 g	0.018 g

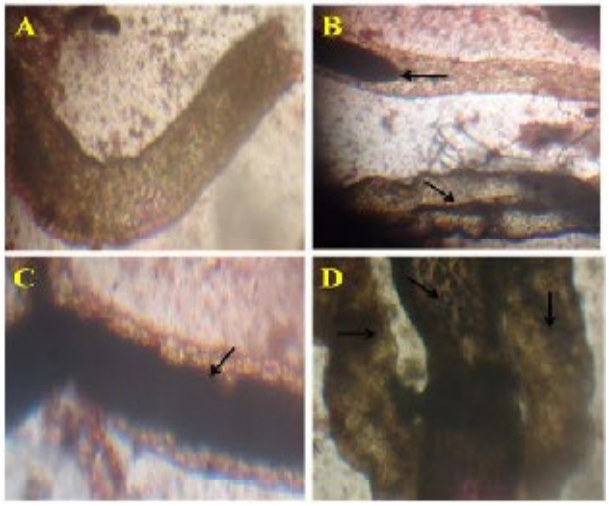

Figure 2: A- Normal second instars larvae midgut, B- Chayote latex inhibition to midgut, C and D- Highly binding latex sample

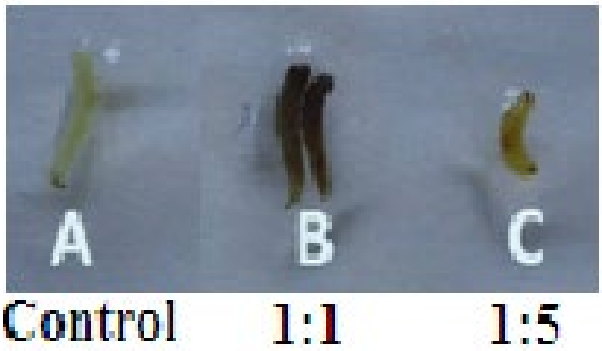

Figure 3: Melon fly second instar larvae diet on various chayote latex sample is significance were observed after 5th hour to 10th hour diet larvae in midgut binding show on A- control, B- latex diet on dead larvae and c- lightly binding latex

activity of enzymes was expressed as µmoles *t*-cinnamic acid formed per minute per mg of protein (µmol min^{-1} mg^{-1} protein).

Tyrosine-alanine lyase (TAL) assay

Tyrosine-alanine lyase activity is determined by the reaction mixture consisting of 0.5 mL of enzyme extract and 150 mM of *L*-tyrosine was adjusted to 3 mL with the extraction buffer. Incubation was done at 30 ± 1°C for 30 min. Tyrosine-alanine lyase activity was determined in terms of coumaric acid at 310 nm. The concentrations of *p*-coumaric acid were determined from the standard curves prepared by plotting absorbance at 290 nm. Specific activity of enzymes was expressed as µmol *p*-coumaric acid formed per minute per mg of protein (µmol min^{-1} mg^{-1} protein).

Cinnamyl alcohol dehydrogenase (CAD):

The activity of cinnamyl alcohol dehydrogenase (CAD) was determined according to Wyrambik and Grisebach [14] by measurement of cinnamyl alcohol produced by the action of enzyme on cinnamaldehyde via oxidation of NADPH.

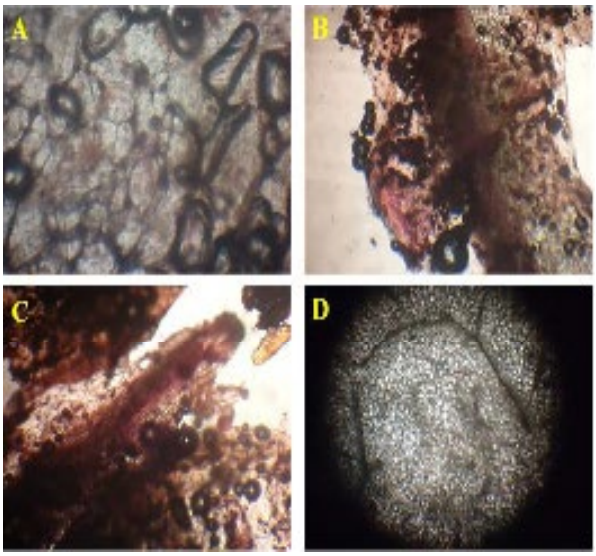

Figure 4: A- Cucumber healthy tissue, B- Cucumber apparent healthy tissue, C- infestation cucumber tissue and D- Chayote healthy tissue

The reaction mixture in a final volume of 3 mL contained 60 µL of 1 mM 600 µL of 1.0 mM NADPH, 600 µL of enzyme extract, 50 µL of 20 mM $ZnSO_4.7H_2O$ and 1150 µL of 0.1 M KH_2PO_4/Na_2HPO_4 buffer (pH 7.6). A negative control containing the reaction mixture components and water in place of cinnamaldehyde was maintained. The linear decrease in absorbance caused by oxidation of NADPH between 0 and 15 min of incubation at 30°C was recorded spectrophotometrically at 340 nm. The activity was expressed as µmoles min^{-1} mg^{-1} protein.

Estimation of total phenolic and flavanoid content

The total phenolic compounds were assayed as described Huda-Faujan et al. [15] in different tissue parts of fruit infested with melon fly. Briefly, the healthy and infected tissues were extracted for phenolics by homogenization using PBS at pH 7.2. 1 mL of the sample was taken to that 1 mL of F-C reagent containing 10 mL of 7% Na_2CO_3 solution was added. Sample was further diluted with 10 mL of distilled water and vertex for 2 h. Later the tubes were read at 760 nm using spectrophotometer. The phenolic content was expressed as mg of Gallic acid equivalent weight from the standard Gallic acid used to prepare standard curve (Figure 5). The total flavonoid contents were determined [16] in various fruit tissues. Briefly, healthy and infected portion of fruits were extracted using PBS at pH 7.2. About 500 uL of sample was diluted with 4ml of distilled water and then added with 0.3 mL of 7% $NaNO_2$ and 0.5 mL of 10% $AlCl_3$. The mixture was incubated for 15 min and was added with 1 M NaOH and 10 mL of distilled water. The absorbance of solution was read at 518 nm and was compared with the standard curve obtained using Catechol. The flavonoid content of the sample was expressed as catechol equivalent weight.

In *vitro* assay for antioxidant potential

Phosphomolybdate assay (total antioxidant capacity)

The Phoshomolybdate activity were followed by Moukette et al. [17], the various cucumbers and chayote fruit tissue against melon fly infestation. Melon fly infested different fruit tissues were extracted using by PBS at pH 7.2. 0.1 mL of extracted sample mixed with 1 mL of the reagent solution of (0.6 M sulphuric acid, 30 mM of PBS and also 4 mM of ammonium molybdate), than incubated at 95°C for 90 min, after sample tubes are cooled to room temperature, the absorbance at 765 nm is recorded. The reducing capacities of the analyzed extracts were expressed in mg of ascorbic acid equivalents/g of dried extract (mg eq AS/g).

Superoxide anion scavenging assay

The super radical anion scavenging activity of measurement was riboflavin nitrogen tetrazolium blue (NBT) and ferric chloride as described by Naima et al. [18]. In this assay, superoxide anion activity from different cucurbits infested fruit tissue were homogenized with PBS pH 7.2, the homogenate sample 500 µL was mixed with 0.5 mL of phosphate buffer (50 mM, pH 7.6). To that 0.3 ml riboflavin (50 mM), 0.25 mL PMS (20 mM) and 0.1 mL NBT (0.5 mM) were added. The mixed samples were incubated at 25°C for 10 min, after incubation absorbance at 560 nm in a spectrophotometer was measured against standard ascorbic acid as a control. Decreased absorbance of the reaction mixture indicated an increased superoxide anion scavenging activity.

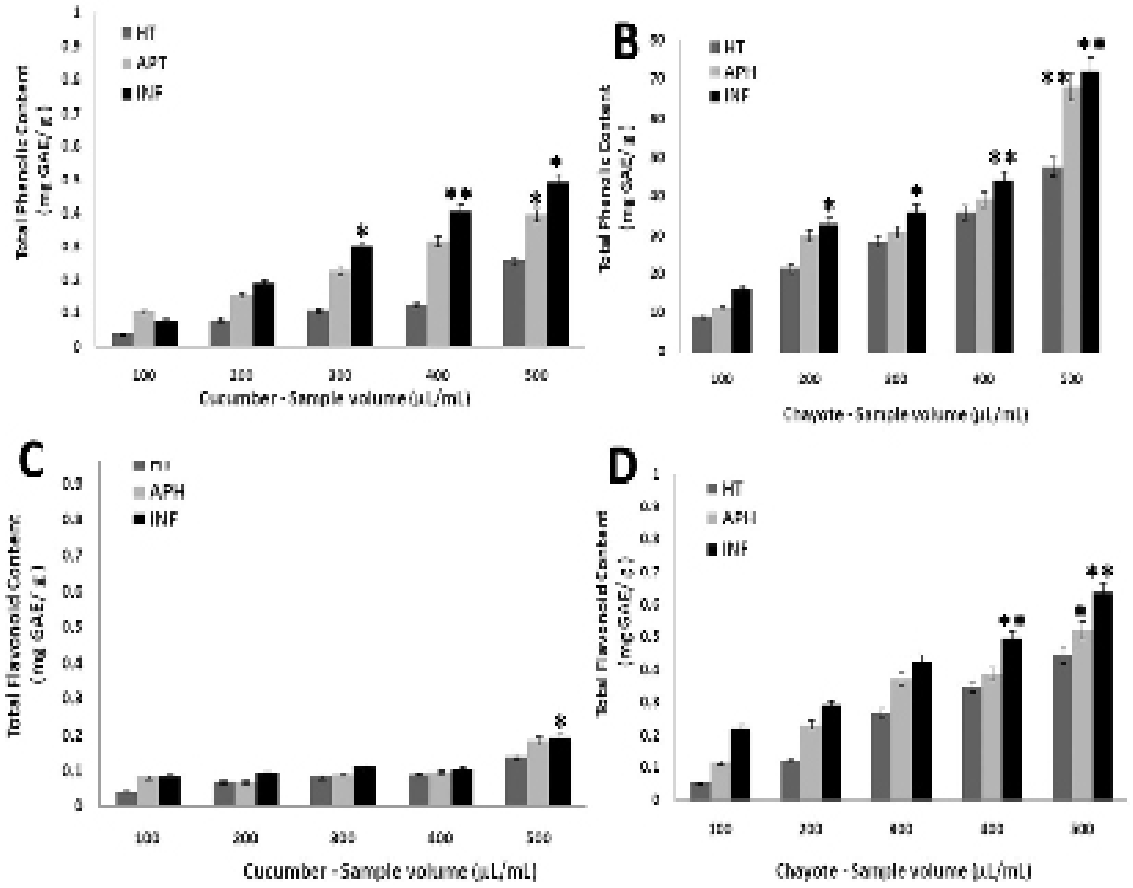

Figure 5: Comparison between non-enzymes activities of cucumber and chayote fruit in phenolic (Cucumber A and B) and Flavanoids (Chayote C and D) content in various tissues in healthy, apparently healthy and infected tissues

Data were represented as mean ± SEM of three independent experiments. Statistical significant values were expressed as *p<0.05 and **p<0.01

DPPH radical scavenging activity assay

The DPPH radical potential scavenging activities in infested, apparent healthy and healthy tissues against melon fly infestation [19]. Briefly, 1 g of fruit tissues were mixed with methanol (90%), the homogenate tissue sample of 0.5 mL was added with 2.5 mL of 200 mM DPPH and kept in dark place for 30 min. The absorbance at 517 nm was measured. Ascorbic acid (ASC) was used as a standard for comparison.

In vivo assay for antioxidant enzymes

The tissues samples (Infected, Apparent healthy and healthy with melon fly infestation) each with 1.0 g were taken and homogenized with 3 mL of ice-cold 100 mM potassium phosphate buffer, pH 6.8, containing 0.1 M EDTA. The mixtures of the sample were filtered using what man no.1 filter paper. Then filtered sample were centrifuged at 16000 rpm for 15 min and supernatant were collected and used for further *in vivo* studies.

Superoxide dismutase (SOD) activity

The assay was carried out according to the procedure described by Beauchamp and Fridovich [20], Briefly the 0.1 mL homogenate samples were mixed with 1.9 mL of phosphate buffer (pH 7.8), followed by addition of 0.5 mL of 2 M methionine, 0.1 mL of 5 M NBT and 0.5 mL of 6 M riboflavin, in a total volume of 3 mL. Illumination of the solution taken in 10 mL beaker was carried out in an aluminum foil lined box, with a 15 W fluorescent lamp for 10 min. The absorbance was measured at 560 nm.

Peroxidase (POX)

The activity of POX was determined in the cucurbitaceous fruits of different tissue samples with melon fly infestation according to [21]. Fruit tissues were extracted with 100 mM potassium phosphate buffer (pH 6.8), about 25 µL of

sample were taken and 2 mL of 50 mM potassium phosphate buffer (pH 6.8) containing 20 mM guaiacol and 20 mM H_2O_2 was added. The samples are mixed and incubated at 30°C for 10 min, following incubation 0.5 mL 5% (v/v) H_2SO_4 was added and the absorbance was read 480 nm.

Catalase (CAT)

Catalase (CAT) activity was determined as described by Biapa et al. [22]. The fruit tissue samples were homogenized and 50 µL of samples were added with 3 ml of 100 mM potassium phosphate buffer pH 7.0 and 20 mM of H_2O_2 as substrate. The decrease in absorbance at 240 nm was measured compared to control without enzyme. The activity was expressed as µmol min^{-1} mg $protein^{-1}$.

Statistical analysis

Data were expressed as Mean ± Standard Deviation (SD). The values were then subjected to one-way ANOVA followed by Turkey's multiple comparison tests for significant difference. The level of significance was considered at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS

Melon fly infestation pronounces phenolic and flavanoids content in cucurbit fruits

The total phenolic and flavonoid contents were estimated in the cucumber and chayote after melon fly infestation. The total phenolic and flavonoid contents are significantly increased in chayote fruit tissue compared to cucumber fruit tissue with melon fly infestation (Figure 2), Chayote infected tissue contains more phenolic content 16.2% compared to APH 11.2% and HT 9.2% and Cucumber APH tissue is 0.106% more phenolic compared INF tissue 0.078% and HT 0.039%. Flavonoids content in cucumber infested tissue is 0.085% and APH tissue 0.082% both are nearly similar compared to HT 0.044%. While in chayote infested fruit tissue content is 0.225% compared to APH 0.117% and HT 0.054%.

Cucurbits exert accelerated ROS detoxifying potential as a defensive response against melon fly

The total antioxidant and defensive capacities of Chayote and Cucumber fruit tissue against melon fly infestation are identified; Cucumber infested fruit tissues having 62.0% activity compared to APH 58.9% and HT 56.01%. Chayote infested fruit tissues are more about 70.52% compared to APH 68.87% and HT 56.01% activity. Superoxide anion radical scavenging activity on infested cucumber fruit tissue and chayote fruit tissue are shown in Figure 6, INF tissue 0.74%, more active compared to APH 0.69% and HT 0.48%. Chayote infestation fruit tissue are highly active compared to cucumber fruit tissue that is 1.32%, in infested fruit tissue compared to APH 1.20% and HT 1.68%. DPPH antioxidant activity on infested cucumber fruit tissue and chayote fruit tissue are having more scavenging as 42.5% compared to APH 42% and HT 30% and chayote are resistance with APH tissue increased activity 65% compared to INF 60% and HT 44%.

Melon fly infestation up regulates antioxidant enzymes in chayote and cucumber

The activation of antioxidative defense systems in plants by biotic stresses is a general phenomenon and probably contributes to increased resistance against a subsequent stress. Therefore in this study the antioxidant enzyme levels after melon fly infestation were evaluated. Results demonstrated that melon fly induced stress has tremendously increased the SOD enzyme activity in the infected and apparent healthy tissue of the chayote fruit by 256% and 174% compared to the normal tissue (Figure 7). The percentage increase in SOD activity in the infected cucumber tissue is very low with 89% to that of healthy tissue. Similarly the POD and catalase specific activity was also pronounced in the cucumber infected tissue with 77% and 68% respectively when compared to control. But cucumber with 285% and 231% of POD and catalase activity in the infected site, it demonstrated a poor elicitation of defense enzymes. It is very obvious from these results that cucumber upon melon fly infection showed reduced activation of antioxidant enzymes in comparison to the Chayote.

Melon fly infestation activates central phenylpropanoid pathway (PPP) enzyme synthesis

The activation of phenylpropanoid pathway enzymes and its secondary metabolites are highly crucial for the plants ability to mount successful defense against invading organisms. Therefore we sought to elucidate the basal expression levels of central PPP enzymes in the chayote and cucumber post melon fly infection (Figure 8). Our results elucidated a significant difference in the level of PPP enzymes in the infected and healthy tissue Chayote, whereas only marginal differences were observed in the cucumber. Melon fly infection lead to apparent doubling of PAL and TAL levels in

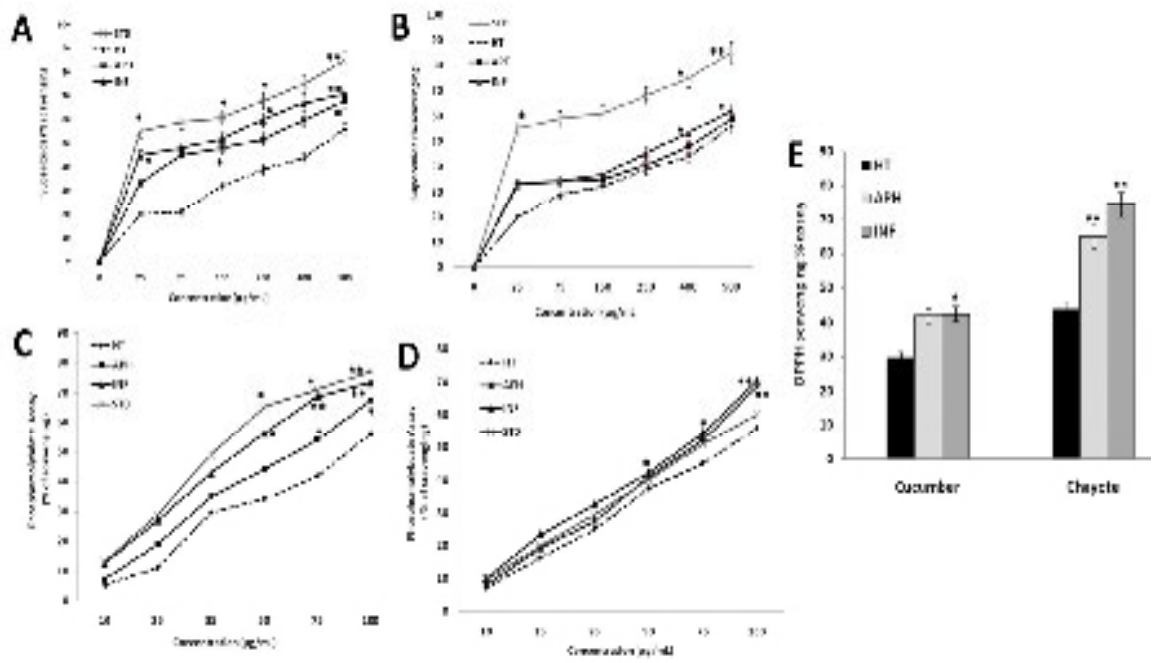

Figure 6: Comparison between antioxidant Activities in A=Phosphomolybdate Assay Cucumber, B=Phosphomolybdate Assay – Chayote, C and D=Superoxide Anion Assay – Cucumber, D=DPPH assay – Cucumber and chayote fruit in healthy, apparently healthy and infected tissues of chayote and cucumber fruits. Data were represented as mean ± SEM of three independent experiments

Statistical significant values were expressed as *p<0.05 and **p<0.01

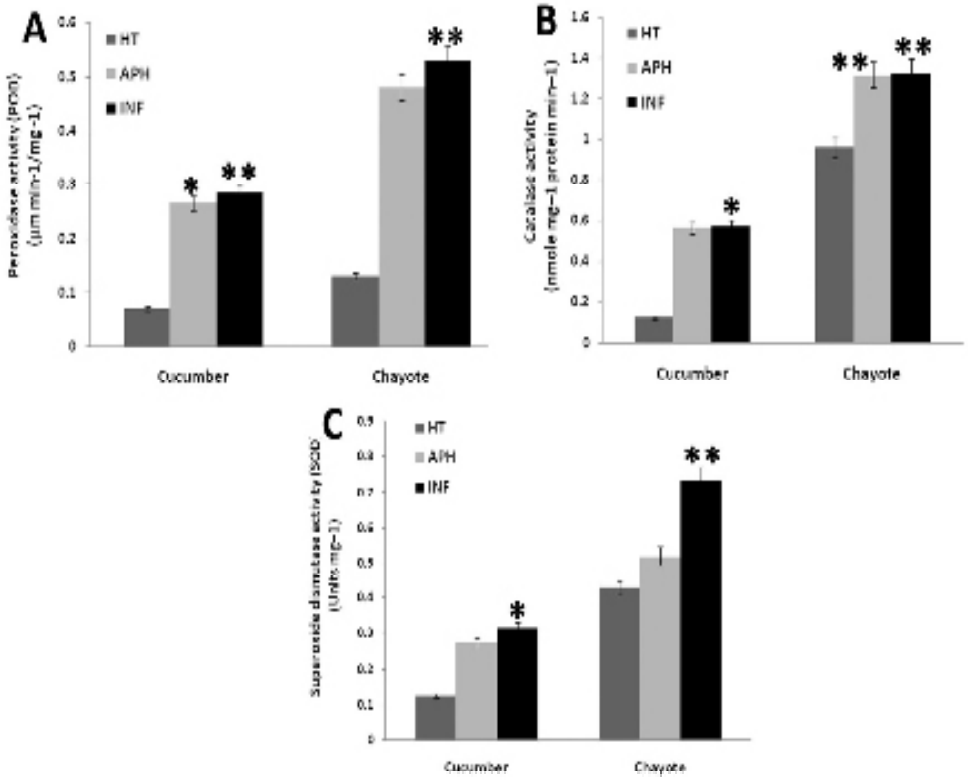

Figure 7: Scavenging antioxidant activity of A=peroxidase (POD), B=Catalyase (CAT) and C=Superoxide dismutase (SOD) cucumber and chayote fruit in various tissues in healthy, apparently healthy and infected tissues

Data were represented as mean ± SEM of three independent experiments. Statistical significant values were expressed as *p<0.05 and **p<0.01

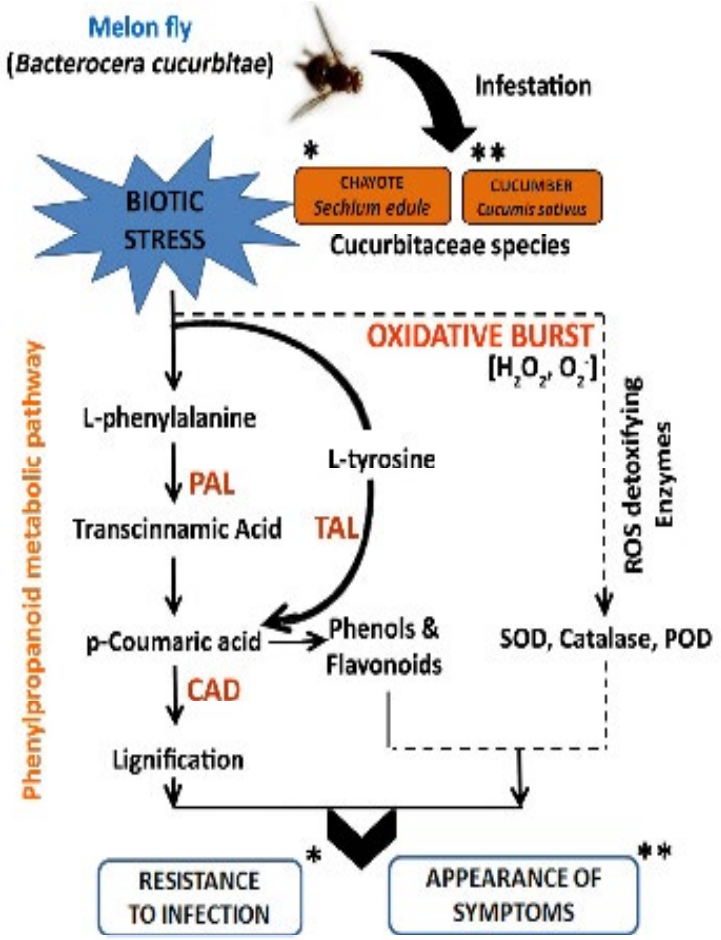

Figure 8: Schematic representation and proposed hypothetical model for the up regulation of phenylpropanoid pathway enzymes and ROS enzymes in response to the biotic stress induced by melon fly infection

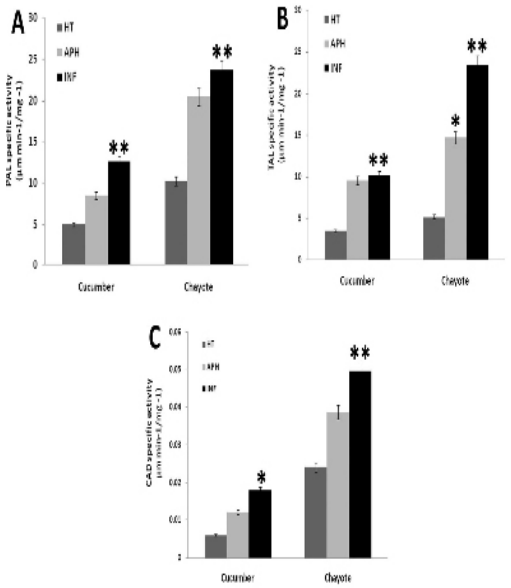

Figure 9: Melon fly infestation triggers Central PPP enzyme activation in cucurbits. Specific activities of (A) phenylalanine ammonia-lyase (PAL), (B) tyrosine ammonia-lyase (TAL), (C) cinnamyl alcohol dehydrogenase (CAD) in healthy, apparently healthy and Infected tissues of chayote and cucumber fruits

Data were represented as mean ±SEM of three independent experiments. Statistical significant values were expressed as *p<0.05 and **p<0.01

chayote as assessed from the infected and apparent healthy tissues (Figure 9). Although cucumber displayed pronounced activity PAL and TAL upon biotic stress, it is lower compared to the chayote. Thus our reports infer that constitutive levels of TAL and PAL were highly accelerated in the infected of the chayote, implicating its resistance, whereas the cucumber with minimal induction of PPP enzymes resulted in susceptibility to *B. cucurbitae* infestation[23].

DISCUSSION

Plants respond to biotic stress by synthesizing the phenylpropanoids which starts with the shikimate pathway. The final products of the shikimate pathway in the aromatic amino acids (phenylalanine, tryptophan, tyrosine), which have essential roles in plant metabolism and serve as precursors for a different of hormones and aromatic secondary metabolites [24]. These secondary metabolites play important roles in the either as local or systemic resistance factors in protecting the plants against various pathogens [25]. *Bacterocera cucurbitae* (Melon fly) is one of the detrimental pests on numerous cucurbitaceous crops. In this study, the responsiveness of cucumber to *B. cucurbitae* was analyses by investigating the levels Phenylpropanoid pathway (PPP) enzymes and antioxidant activity in the infected and healthy tissues. Our early reports indicate that chayote has developed a marked resistance to *B. cucurbitae* infestation by eliciting and accumulating the PPP enzymes upon infection [11]. Due to the reports pertaining to the potential stress adaptation of chayote, the study was conducted by evaluating the metabolic changes that occur in cucumber in comparison with the chayote post *B.cucurbitae* infection. The general Phenylpropanoid pathway leads from phenylalanine to coumaroyl-CoA and is initiated from the enzyme phenylalanine ammonia-lyase [26]. This enzyme is one of the most intensively studies in the plant secondary metabolism, because of the key role it plays in Phenylpropanoid biosynthesis [27]. Our reports indicate that *B. cucurbitae* infested cucumber and chayote showed an increased PAL enzyme activity with the percentage activity being higher in the chayote. Analogous to the PAL activity, tyrosine alanine lyase (TAL) activity which deaminates tyrosine to form p-coumarate [27] was also found to be increased in the infected tissues. Cinnamyl alcohol dehydrogenase (CAD) catalyses the reduction of hydroxycinnam aldehydes to hydroxycinnamyl alcohols which is considered to be an indicator of lignin biosynthesis because of its specific role at the end product of the monolignol biosynthetic pathway. CAD is also expressed in response to stress, pathogen elicitors, and wounding. CAD is therefore regulated by both developmental and environmental pathways [28]. Our study too has showed a pronounced CAD activity upon the *B. cucurbitae* infection in the infected site in cucumber and chayote compared to its healthy tissue.

Phenylpropanoid metabolism comprises a complex series of branching biochemical reactions which provide the plant with a host of important phenolic compounds and flavonoids [26]. Phenolic acids, apart from their known role in resistance to insect pests, they also confer resistance to infection by a large number of pathogens, including fungi, bacteria, and viruses in plants. This kind of biotic stress induces the increased synthesis of phenolic acids, which are then incorporated to the cell wall of plants [25]. In this study, *B. cucurbitae* infected cucumber tissues were assayed for the total phenolic content and flavonoid content. Results inferred an increase in the phenolic content and flavonoid content in the infected tissue and apparently healthy tissue. The results are in correlation with increased PAL enzyme activity which is the precursor for the numerous phenolic and flavanoids. This could be probably due to the development of the resistance against an occurring infection.

One of the first and most important protection and signaling mechanisms that is induced in infected plants is the production of ROS and consequently the accumulation of ROS-scavenging enzymes and metabolites [24]. The high correlation coefficient between phenolic acids content and antioxidant capacity in vegetables reveals that they play a main role in the bioactive properties of these plant products [25]. The antioxidative processes protect plant tissues from lipid peroxidation processes around the infection sites and thereby protect them from the spreading of necrotic lesions [23]. In this study the levels of SOD, catalase and antioxidant capacity of the melon fly infected tissue were evaluated to understand the role of protective antioxidative systems in plant response to pathogen attacks, particularly in the induction of SAR. The study found that there was a substantially increased SOD, catalase and GSH in the *B. cucurbitae* infected and apparently healthy tissues of cucumber than the normal tissue. The enhanced activity of catalase and SOD might be considered as an evidence for the stress induced H_2O_2 and O_2 generation. As a confirmation the infected and apparently healthy tissues demonstrated an increased ROS scavenging activity as evidenced from total antioxidant activity, superoxide scavenging activity and DPPH assay. Though the level of antioxidant enzyme activity was up regulated, it was lesser than the chayote activity in response to the infection, which would explain its high level of resistance to the melon fly infestation as reported earlier [11]. But experimental evidences have shown

that SAR generally means resistance against necrosis (symptom expression) and not necessarily against pathogens [23]. This could be the reason for the susceptibility and low level of resistance in some plants against various biotic stresses despite the development of SAR.

CONCLUSION

The study has provided evidences that a biotic stress induced by the well-known pest, *Bacterocera cucurbitae* (Melon fly) infection has potentially accelerated biochemical and defense systems in cucumber and chayote. The enzymes especially PAL and TAL belonging to phenylpropanoids pathway (PPP) which is the precursor for phenolic and flavanoids were notably up regulated in the infected site of the Cucumber and Chayote tissue [29-31]. In relevance to this, the infected tissue of both cucumber and chayote showed an increased total phenolic, total flavanoids and antioxidant enzyme activities (SOD, POD and catalase). Taken together, it is concluded that *B. cucurbitae* infection has increased various enzymes and ROS scavenging antioxidant systems in order to combat stress. Although this study has provided evidences and insights about the accumulation and modulation of defense related enzymatic activities in cucumber upon biotic stress, an in depth study involving the gene expression analysis in the infected and healthy tissue is necessary, which would provide a precise mechanism of action.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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