Available online at www.easletters.com

ISSN No: 2349-2864

Entomology and Applied Science Letters, 2015, 2, 1:42-46

Total phenol content in thrips infested rice leaves of Khasi Hills

Magdalene Kharbangar*, Sudhanya Ray Hajong and Sudipta Choudhury

Entomology Laboratory, Department of Zoology, North Eastern Hill University, Meghalaya, Shillong

Correspondence: mag.kbangar@gmail.com

(Received: 12/8/14) *(Accepted:24/12/14)*

ABSTRACT

Thrips infestation in rice leaves causes a considerable leaf yield loss and quality. An attempt was made to know the changes in secondary metabolites constituents (viz, total phenols) in normal and thrips infested cultivated rice leaves of Mawlai (25º 60' N 91º 90' E), and Nongjri (25º 74' N 91º 90' E) of Khasi Hill. The amount of total phenols, were analyzed using a spectrophotometric technique, based on Folin-Ciocalteau reagent showed significant variation in phenol content from both the sites as compared to the normal rice leaf. Gallic acid was used as standard compound and the total phenols were expressed as mg GAE/g leaf extract. The total phenols varied from 18.89 ± 0.39 to 28.55 ± 0.98 mg GAE/g leaf ext ract in normal leaf extracts. A higher phenolic content was found in thrips infested leaf extract varying from 287.29 ± 5.06 to 340.3 ± 0.72 mg GAE/g leaf extract in both the studied sites.

Keywords: Rice leaf, normal, infested, phenolic content, secondary metabolites

INTRODUCTION

Rice (*Oryza sativa* L.) is the most grown cereal crop in the world. It is used as staple food for more than three billion people in Asia [1]. It also provides 50 to 80% of daily calorie in Asia [2]. The global production of rice includes about more than 508,697,332 metric tons per year [3]. More than one million herbivorous insect species have been described so far, with different feeding strategies leading to different quantity and quality of mechanical damage on plant tissue. Two thirds of all known herbivores are leaf-eating beetles (Coleoptera) or caterpillars (Lepidoptera) that cause damage with mouthparts evolved for chewing, snipping or tearing [4]. Leaf miners feed on the soft tissue between the epidermal cell layers, while piercing-sucking herbivores, such as spiders and thrips, have a tube-like structure which are used to suck the liquid content from lateral cells. Phloem-suckers such as aphids, whiteflies and other Hemiptera have special stylets that are inserted between the cells into the phloem [5].

Plants and insects have been living together for more than 350 million years. In co- evolution, both have evolved strategies to avoid each other's defense systems. This evolutionary arms race between plants and insects has resulted in the development of an elegant defense system in plants that has the ability to recognize the non-self molecules or signals from damaged cells, much like the animals and activates the plant immune response against the herbivores [6, 7]. There are inter-connections that exist between distinct and opposing signaling response pathways for defense against pathogens and insect herbivores and there also appear to be multiple response pathways invoked, depending on the specific stress context towards plants [8-16].

Plants confront the herbivores both directly by affecting host plant preference or survival and reproductive success (direct defense), and indirectly through other species such as natural enemies of the insect pests (indirect defense) [6, 17, 18]. Direct defenses are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface of the plants (e.g., hairs, trichomes, thorns, spines, and thicker leaves) or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinones that either kill or retard the development of the herbivores [19]. Indirect defenses against insects are mediated by the release of a blend of volatiles that specifically attract natural enemies of the herbivores and/or by providing food (e.g., extra floral nectar) and housing to enhance the effectiveness of the natural enemies [18]. Plants produce a large variety of secondary products that contain a phenol group, a hydroxyl functional group on an aromatic ring which plays an important role in the plants defense system against pests and diseases [20].

Phenolic compounds are a diverse group of secondary metabolites and are widespread in plants [21]. Studies on the activity and role of phenolic compounds have shown a wide range of effects on insects. Early research stressed the growth reducing properties of phenolics towards different insects, and phenolics were thought to function as broad-spectrum defenses to which insects would be unable to develop counter-adaptations [22-24]. Phenolic compound can have different effects on different insects [25]. Many plants respond to herbivory and other biotic or abiotic stresses by producing elevated levels of phenolics. For example, Fall armyworm, *Spodoptera frugiperda*, is a highly polyphagous insect that has been recorded on over 60 plant species from at least 23 plant families [26, 27]. However, its favoured hosts are grasses, and it is an important pest of several graminaceous crops, including rice, corn, sorghum and forage grasses [28, 29]. This insect commonly encounters high concentrations of phenolic compounds when feeding on grasses. Several compounds, namely ferulic acid and *p*-coumaric acid, are commonly found in plants and are particularly common in grasses [30, 31]. Studies on total phenols in *O. sativa* were measured following infestation by the Asian gall midge, *Orseolia* [32]. Minor differences in chemical structure of phenolics may significantly alter the biological activities on insects. The differences in biological activity may be due to differences in affinity for specific target sites or to alterations in interactions with detoxifying enzymes [33, 34].

In our earlier study we found that in Khasi hills a total of 5 species of thrips were observed viz, *Stenchaetothrips biformis* (Bagnall), *Haplothrips tenuipennis* (Bagnall), *Haplothrips ceylonicus* (Schmutz), *Anaphothrips sudanensis* (Trybom) and *Bolacothrips indicus* (Ananthakrishnan) infesting rice crops [35]. The present study attempts to determine the phenol contents in infested and uninfested rice leaves so as to determine the effect of infestation on secondary metabolites which may be involved in defense.

MATERIALS AND METHODS

1.1 Study areas

The study site was carried out at rice fields located at Mawlai (Near University Campus) (25°60' N 91°90' E) and Nongjri (25° 74' N 91° 90' E) in Khasi Hills, Meghalaya. Both infested and uninfested rice leaves were collected from the fields in the year 2010 to determine the total phenolic contents.

2.1 Sample preparation

The leaves of *Oryza sativa* L. were collected by hand and cleaned with tap water. 1.0g of sample were weighted exactly and grinded with a pestle and mortar. These were then extracted in 10 times volume using 80% ethanol (v/v). The mixture was then centrifuged at 10,000rpm for 20 minutes. The supernatant was saved and the residue was re-extracted with five times the volume of 80% ethanol. The supernatant was again centrifuged, pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water (5mL).

2.2 Determination of Total phenolic compounds

Total phenolic compounds (TPC) of samples were determined using Folin-Ciocalteau (FC) assays as described by Malick and Singh, 1980. Extracted samples obtained were pipetted out in triplicates (0.2 to 2mL) into test tubes. The volume of the solution in each test tube was mixed with distilled water to make the solution to 3mL. To each test tube 0.5mL of Folin-Ciocalteau reagent was added after 3 minutes, 2mL of 20% Na_2CO_3 solution was then added to each tube. The solution was thoroughly mixed and the tubes were placed in boiling water for one minute. The test tubes were then cooled and absorbance was measured at 650nm against a reagent blank using UV-visible spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE)/g of sample [36].

2.3 Statistical analysis

Results obtained were reported as mean + SD (mg GAE/g of extract) in triplicate measurements.

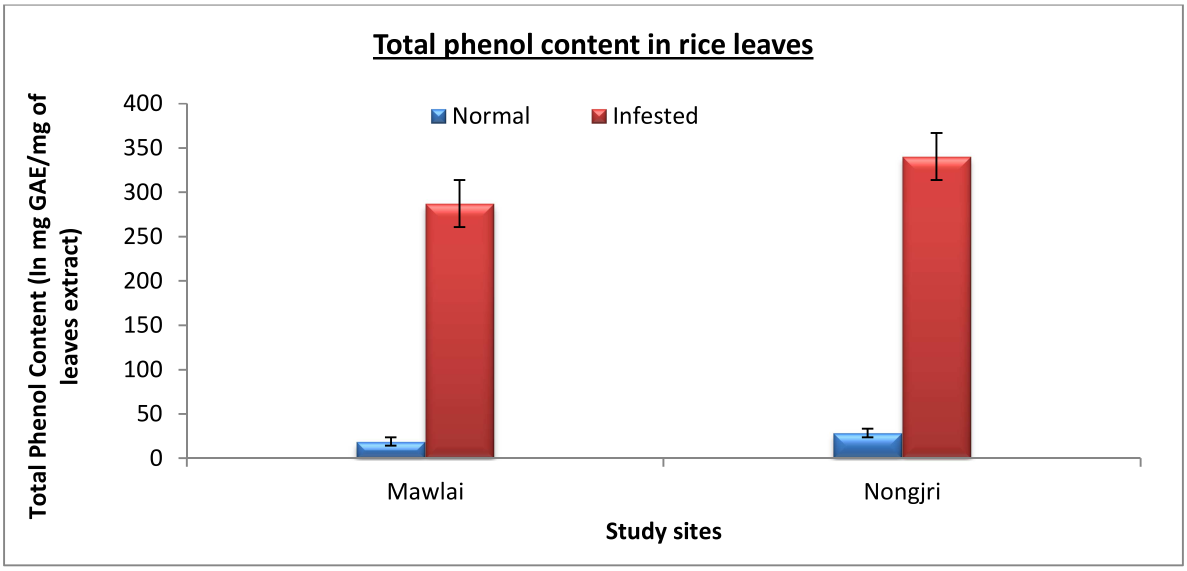
RESULTS AND DISCUSSION

The average quantity of the total phenolic compounds found in normal and infested rice leaves extracts is shown in (**Table 1**). The amount of total phenolics content in rice leaf in varieties of rice collected from Mawlai and Nongjri were in the range of 18.89 – 287.29 mg GAE/mg of extract and 28.55 – 340.3 mg GAE/mg of extract respectively from both normal and infested leaves. The results revealed the highest total phenolic content in infested leaves of both the sites.

Table 1: Total phenolic content in normal and infested rice leaves from Mawlai and Nongjri site

Study sites	Types of leaves	Mean Total Phenol content ±SD (mg GAE/g of leaves extract)
Mawlai	Normal	18.89 ± 0.39
	Infested	287.29 ± 5.06
Nongjri	Normal	28.55 ± 0.98
	Infested	340.3 ± 0.72

Fig 1: Determination of Total Phenol Content in rice leaves



In agronomy, an insect is classified as a pest if the damage it causes to a crop is sufficient to reduce the yield or the quality of the harvested product by an amount that is unacceptable to the farmer [37]. Injury is the effect of pest activities on host physiology, which is usually deleterious, while damage is a measurable loss host yield quantity or quality [38]. Cereals have been important in agriculture ever since man started to cultivate crops. The effects of leaf-feeding by terebrantian thrips on rice crops can be much more obvious and serious and heavily infested leaves of all ages shrivel, become discoloured and brittle, and eventually fall, especially in hot dry weather [39]. After more extensive feeding, when the plant tissue showed obvious external signs of damage, including silvering, the internal cell structure was completely disrupted, the epidermal cells, especially the bulliform cells get shrivelled and distorted, which is a sign of extreme desiccation [40].

A plant defense is categorized as anti-nutrition and toxicity. Anti-nutrition can occur as both pre-ingestion to limit food supply and post-ingestion to reduce nutrient value to the attacking insect and toxicity is via, chemical disruptions to the attacking insect by specific plant traits through secondary metabolites. Different plant secondary metabolites suppress the herbivore’s growth and development via different mechanisms. Some flavonoids are inhibitors of regulatory enzymes such as calcium dependent ATPase [41]. Lignin and other phenolics can strengthen cell walls and therefore can be anti-nutritional [42, 43]. Some phenolics and sesquiterpenes along with other volatiles can repel herbivores from oviposition on host plants [44, 45]. Other comprehensively described compounds act as feeding deterrents [46].

The present result obtained is related to the study done by Krasaetep J. *et al.* on total phenolic contents in leaf of some Thai Rice cultivars where TPC was highest in the milking stage of rice [47], other study by Moure *et al. on Cynometra cauliflora* showed higher TPC compared to *Garcinia atrovirdis* [48] and the total phenolic contents determined in the leaves of *Rosa* species were found to range from 57 - 152 mg GAE/g dw [49] which is in line with the study's results. Therefore, the result supports the hypothesis that phenolics are involved in the defense against thrips leading to resistant genotypes.

CONCLUSION

The experimental results indicate that such increases in total phenols are considered elements of induced resistance in hosts against herbivory. However, despite high levels of phenols in infested rice leaves, thrips infestations were observed to an appreciable extent. This could possibly be attributed to the proportionately high levels of readily utilizable primary substrates. It is also possible that the prevalence of less toxic phenolic acids may contribute to the ability of thrips to infest the host.

Acknowledgement

The authors are thankful to the Head, Department of Zoology, North Eastern Hill University, for providing assistance in necessary laboratory facilities during this work, UGC Research Fellowship in Science for Meritorious Students for financial support and to Prof. R. Varatharajan, Department of Life Sciences, Manipur University.

REFERENCES

[1] P. Bhattacharjee, R. S. Singhal, P. R. Kulkarni, *Int J Food Biophys*, **2002**, 49, 115-123
[2] G. S. Khush, *Pl Mol Bio*, **2005**, 59, 1-6
[3] Food and Agriculture Organization (FAO) (2009-2010), International Exhibition to Promote rice Import & Export, Abu Dhabi National Exhibition Centre, UAE. **2012**
[4] L. M. Schoonhoven, T. Jermey, J. J. A van Loon, Insect Plant-biology: From Physiology to Evolution; Chapman and Hall: London, UK. **1998**
[5] D. F. Houlihan, *J. Zool*, **1969**, 159, 249–267
[6] G. A. Howe, G. Jander, *Annu Rev Plant Biol*, **2008**, 59, 41-66
[7] J. D. Hare, *Annu Rev Entomol*, **2011**, 56, 161-80
[8] W. Agosta, Bombardier beetle and fever trees: A close up look at chemical warfare and signals in animals and plants. Addison-Wesley, Reading, MA. **1996**
[9] M. G. Barbour, J. H. Burk, W. D. Pitts, Terrestrial plant ecology, Second edition, Benjamin/Cummings, Menlo Park, Calif. **1987**
[10] R. M. Bostock, *Physiol Mol Plant P*, **1999**, 55, 99-109.
[11] R. M. Bostock, R. Karban, J. S. Thaler, P. D. Weyman, D. Gilchrist, *European J Plant Pathol*, **2001**,107, 103-111
[12] R. Hell, *Planta*, **1997,** 202, 138-148
[13] B. P. H. J. Thomma, K. Eggermont, I. A. M. A. Penninckx, B. Mauch-Mani, R. Vogelsang, B. P. A. Cammue, W. F. Broekaert, *Proc Nat Acad Sci*, **1998**, 95, 15107-15111
[14] P. Vijayan, J. Shockey, C. A. Levesque, R. J. Cook, J. Browse, *Proc Nat Acad Sci*, **1998**, 95, 7209-7214
[15] R. H. Whittaker, P. P. Feeny, *Sci*, **1971**, 171, 757-770
[16] A. Kusnieczyk, P. Winge, H. Midelfart, W. S. Armbruster, J. T. Rossiter, A. M. Bones, *J Exp Bot*, **2007**, 58(10), 2537-2552
[17] N. Dudareva, F. Negre, D. A. Nagegowda, I. Orlova, *Crit Rev Plant Sci*, **2006**, 25, 417-40
[18] G. I. Arimura, K. Matsui, J. Takabayashi, *Plant Cell Physiol*, **2009**, 50, 911-23
[19] M. E. Hanley, B. B. Lamont, M. M. Fairbanks, C. M. Rafferty, *Perspec Plant Ecol Evol Syst*, **2007**, 8,157-78
[20] N. Wuyts, D. De waele, R. Swennen, *Plant Physiol Biochem*, **2006**, 44, 308-314
[21] P. G. Waterman, S. Mole, Analysis of phenolic plant metabolites. Blackwell Scientific Publications, Oxford, UK. **1994**
[22] P. F. Feeny, *Ecol*, **1970**, 51, 565-581
[23] P. F. Feeny, *Rec. Adv. Phytochem*, 1976, 10, 1-40
[24] D. F. Rhoades, R. G. Cates, *Rec. Adv. Phytochem*, **1976**, 10, 168-213
[25] A. Ikonen, , J. Tahvanainen, H. Roininen, *Chemo ecol*, 2001, 12(3), 125-131
[26] D. R. Johnson, J. J. Kimbrough, M. L. Wall, *Coop. Ext. Serv. EL*, **1987**, 330, 1-15

[27] D. P. Pashley, *Entomol*, **1988**, 71, 227- 233
[28] N. T. Chang, *Chinese J. Econ. Entomol*, **1986**, 6(2), 163-175
[29] D. P. Pashley, Causes of host-associated variation in insect herbivores: an example from fall armyworm., in: Kim, K.C. and B.A. McPheron (eds.), Evolution of insect pests. John Wiley and Sons, Inc., New York. **1993**, pp 351-359
[30] D. Classen, J. T. Arnason, J. A. Serratos, J. D. H. Lambert, C. Nozzolillo, B. J. R. Philogene, J. *Chem. Ecol*, **1990**, 16, 301-315
[31] H. Wu, T. Haig, J. Pratley, D. Lemmerle, M. An, *J Chem Ecol*, **2001**, 27, 125-135
[32] S. Amudhan, U. P. Rao, J. S. Bentur, *Current Sci*, **1999**, 76(12), 1577-1580
[33] M. P. Ayers, T. P. Clausen, S. F. MacLean, A. H. Redman, P. B. Reichardt, *Ecol*, **1997**, 78, 1696-1712
[34] R. L. Lindroth, S. S. Peterson, *Oecologia* , **1988**, 75, 185-189
[35] M. Kharbangar, S. R. Hajong, S. Choudhury, *J Entomol Zool Stud*, **2013**, 1(6), 100-104
[36] C. P. Malick and M. B. Singh. In: Plant Enzymology and Histo Enzymology, Kalyani Publishers, New Delhi. **1980**, pp 286
[37] D. Dent, Insect pest management, 2nd ed. CAB international, Wallingford, UK. **2000**
[38] L. P. Pedigo, Entomology and pest management, 3rd ed. Prentice- Hall, New Jersey. **1999**
[39] T. Lewis, Thrips, their biology, ecology and economic importance, Academic Press London and New York. **1973**
[40] K. Esau. In: Anatomy of seed plants, New York & London, Wiley. **1961,** pp 376
[41] B. K. Salunke, H. M. Kotkar, P. S. Mendki, S. M. Upasani, V. L. Maheshwari, *Crop Prot.* **2005**, 24: 888-893
[42] S. E. Brodeur-Campbell, J.A. Vucetich, D. L. Richter, T. A.Waite, J. N. Rosemier, C. J. Tsai, *Environ Entomol*, **2006**, 35, 1696-1701
[43] F. C. Schroeder, M. L. del Campo, J. B. Grant, D. B. Weibel, S. R. Smedley, K. L. Bolton, J. Meinwald, T. Eisner, *Proc Nat Acad Sci*, USA, **2006**, 103, 15497-15501.
[44] C. M. DeMoraes, M. C. Mescher, J. H. Tumlinson, *Nature*, **2001**, 410, 577-580
[45] D. P. W. Huber, J. Bohlmann, The role of terpene synthases in the direct and indirect defense of conifers against insect herbivory and fungal pathogens, *Multigenic and Induced Systemic Resistance in Plants* (eds. S. Tuzun and E. Bent), Springer-Verlag, New York. **2006**, pp 296-313.
[46] O. Koul, Insect Anrifeedants, CRC Press, Boca Raton, FL. **2005**
[47] J. Krasaetep, M. Nakornriab, D. Puangpronpitag, *Int J Appl Chem*, **2011**, 7, 285 – 296
[48] A. Moure, D. Franco, J. Sineiro, H. Domĭngguez, M. J. Nūňez, J. M. Lema, *Food Res Int*, **2001**, 34(2), 103-109
[49] R. Nowak, U. Gawlik-Dziki, *Z Naturforsch*, **2007**, 62, 32- 38