ISBN: 978-93-48091-58-1

16. Phytochemical Analysis of Food Plants Adopted by Rearers for Ericulture in Kokrajhar District, BTR, Assam

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Abstract:

Differences in phytochemical constituents of different food plants alter the economic parameters of insects. Silk rearers of Kokrajhar district have adopted various plant species for rearing of eri silkworm. Among these adopted plant species, phytochemical analysis of Ficus racemosa and Oroxylum indicum was carried out. Quantitative estimation of the aqueous crude leaf extract of both the plant species shows presence of different phytochemical constituents viz; phenol, flavonoid, tannin and saponin along with protein and carbohydrate. Phenol (68.20±0.27 µg/mg), flavonoid (69.46±0.64 µg/mg), tannin (26.89±1.05 µg/mg), saponin (87.45±1.09 µg/mg) and carbohydrate (34.09±0.84 µ/mg) content in Ficus racemosa was recorded highest. On the other-hand, protein (69.22±0.96 µg/mg) content in Oroxylum indicum was recorded more between the species. The use of these plant species within Kokrajhar district is restricted to some rearers only but further may be adopted as potential alternative host plants for Eri silkworm due to their rich phytochemical composition.

Keywords:

Food plants; Eri rearing; phytochemical; growth; development.

16.1 Introduction:

Sericulture is an agro-based cottage industry that provides livelihood opportunities to the people of rural and marginalized societies (Unni *et al.,* 2009). The multivoltine saturniideri moth species, *Samia ricini* is mostly reared by the people of North-East India. It is regarded as ‘peace silk’ as during spinning pupae inside cocoons is not killed (Satarupa, 2016). It is also called as ‘poor men’s silk’ due to its low cost and high yield nature (Patil and Savanurmath, 1989). Besides having all these potentialities, the industry has so many issues that deteriorate the overall crop and yarn production of *S. ricini*. Ericulture is mostly affected by, improper management and rearing techniques, deprivation of food, seasonal scarcity of host plant leaves, disease infestation, fluctuations in temperature and relative humidity, rainfall and many other biotic and abiotic factors (Unni *et al.*, 2009; Rani *et al.*, 2016; Joncy and Priyadharshini, 2018). Nutrition is a key factor that has direct impact on overall grainage performance of eri silkworm. Proper nutrition surplus the growth and development of eri larva, shorten larval and pupal life and promotes efficient emergence, egg laying and hatching. There is a strong correlation between nutrition of silkworm and host plant. Types and supplied amount of host plant leaves determine the nutritional profile of eri silkworms.

These factors not only maintain nutritional status but also provide immunity to the silkworms to withstand any kind of harsh condition and disease infestation (Siva-Jothy and Thompson, 2002; Yang *et al.,* 2008). Being polyphagous in nature, lots of plant species can be used for rearing of eri silkworms. It includes; *Ricinus communis, Heteropanax fragrans, Manihot esculenta, Gmelina arborea, Evodia fraxinifolia, Ailanthus grandis, Carica papaya* etc. However, out of many host plant species, erirearers mostly preferred leaves of *Ricinus communis* and*Heteropanax fragrans* for rearing of eri silkworms (Kumar and Elangovan, 2010). But due to seasonal scarcity, it is not possible for the erirearers to raise silkworm in *R. communis* throughout the year. Moreover, lack of proper *H. fragrans* plantation also limits ericulture for most of the rearers. That is why; erirearers relay on other plant species that are available within their locality.

Kokrajhar district of Assam is home to many ecoraces and pure line strains of *S. ricini*. Ericulture in Kokrajhar district is one of the important livelihood opportunities for the inhabitants; mostly for the schedule tribe communities. *R. communis*, *H. fragrans* and *M. esculenta* are widely used host plants by the erirearers of Kokrajhar district. But lack of proper plantation site and seasonal scarcity makes erirearers to adopt other wild plants for rearing. *Oroxylum indicum* (Family: Bignoniaceae) and *Ficus racemosa* (Family: Moraceae) are two such kind of alternate food plants adopted by few erirearers of Kokrajhar district, mostly for winter crops. As already mentioned, nutrition is a key factor for the growth and development of eri silkworms. Nutrition comes from food that is supplied to silkworms. Different food plants contain various phyto-nutrients that are essential for growth and development of silkworms (Singh and Das, 2006). Plant foliage also contain different types of secondary metabolites viz; phenol, alkaloid, flavanoid, tannin, saponin and steroids (Changmai*et al.,* 2015). These secondary metabolites are capable of attracting silkworms for consumption of foliage, perform different physiological and metabolic activities within the body and have contributed to the inhibitory and immune response of the silkworm. Grainage performances along with survivability and immunity of eri silkworm changes according to the food plant consumed (Raychaudhury, 1974). There are differences among host plants in terms of nutrient and phytochemical composition. Again, the amount of foliar nutrients and phytochemical constituents show significant variation within the same species in terms of habitat, seasonality, soil property, environmental condition, cultivation techniques and management processes (Das and Das, 2003). Therefore, selection of food plant with proper scientific analysis on nutrient and phytochemical constituents are necessary for successful ericulture. Therefore, the present study was conducted to analyze foliar protein and carbohydrate contents as well as some important phytochemical constituents of *O. indicum* and *F. racemosa*.

16.2 Materials and Methods:

Collection of plant leaves and preparation of aqueous extracts: Fresh leaves of *Ficus racemosa* and *Oroxylum indicum* were collected from nearby area of Kokrajhar town through proper permission. Collected leaves were washed thoroughly and then dried in shade for 4-5 days. After complete drying, these were grounded to fine powder. In order to prepare aqueous leaf extract, method described by Mbaebie *et al.,* 2012 was followed with slight modification. Lyophilization at -50^0C was carried out in order to get aqueous plant extract. Plant leaf extracts were then stored at -20^0C for further use.

Phytochemical analysis of the plant extracts: Phytochemical constituents viz; phenol, flavanoid, tannin, saponin along with carbohydrate and protein content was considered for quantitative estimation. For this, different methods described by different researchers were followed with slight modifications. Freeze dried aqueous plant extract was reconstituted with water in a ratio of 1:1 in order to prepare a stock solution, diluted then after and different concentrations were prepared for quantitative estimation. Three replicates (n=3) were taken for each concentration and values were represented as Mean±SD.

Total Phenol Content (TPC): Total phenol content was estimated following the methods described by Singleton *et al* (1999) and Ordonez *et al* (2006). Gallic acid is used as a reference chemical. Values were measured spectrophotometrically at 760 nm and expressed as µg gallic acid equivalent (GAE)/mg plant extract.

Total Flavanoid Content (TFC): Total flavanoid content was estimated following the methods described by Woisky*et al* (1998) and Ordonez *et al* (2006). Quercetin is used as a reference chemical. Values were measured spectrophotometrically at 420 nm and expressed as µg quercetin equivalent (QE)/mg plant extract.

Total Tannin Content (TCC): Total tannin content was estimated following the methods described by Katoch (2011) and Poudel and Rajbhandari (2020). Tannic acid is used as a reference chemical. Values were measured spectrophotometrically at 700 nm and expressed as µg tannic acid equivalent (TAA)/mg plant extract.

Total Saponin Content (TSC): Total saponin content was estimated following the methods described by Goel *et al* (2012) and Le *et al* (2018) using quillaja saponin as reference chemical. Values were measured spectrophotometrically at 544 nm and expressed as µg quillaja saponin equivalent (QS)/mg plant extract.

Total Carbohydrate Content (TCC): Total carbohydrate content of all the plant extracts was estimated following the Anthrone method (Ludwig *et al.*, 1956). Values were measured spectrophotometrically at 630 nm and expressed as µg carbohydrate/mg plant extract using the standard curve of Glucose.

Total Protein Content (TPC): Total protein content of all the plant extracts was estimated following the Lowry method (Lawry *et al.*, 1951). Values were measured spectrophotometrically at 660 nm and expressed as µg protein/mg plant extract using the standard curve of Bovine Serum Albumin (BSA).

16.3 Results and Discussion:

By following the standard methods to quantify the phytochemical constituent of *O. indicum* and *F. racemosa*, it was found that both the plants are good source of phenol, flavanoid, tannin, saponin, carbohydrate and protein. Standard graphs along with histograms representing the quantity of phytochemical constituents of *O. indicum* and *F. racemosa* are described below:

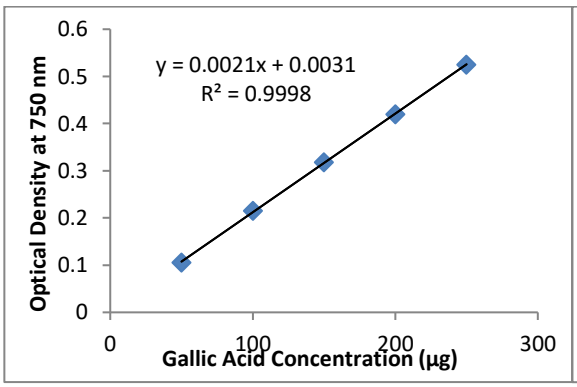

Figure 16.1: Standard graph for Gallic acid

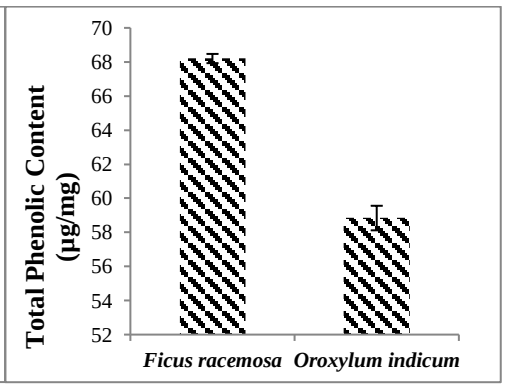

Figure 16.2: Total phenolic content (µg/mg)

Figure: Total Phenol Content (TPC)

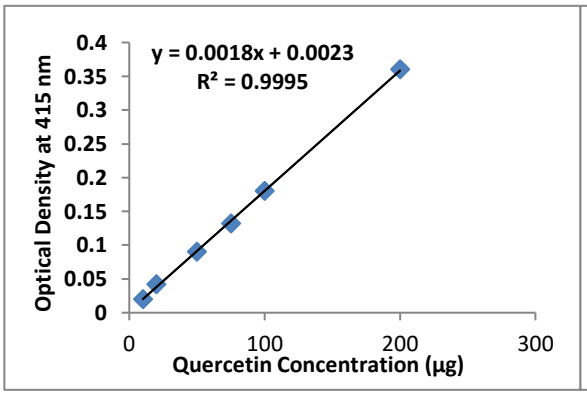

Figure16.3: Standard graph for Quercetin

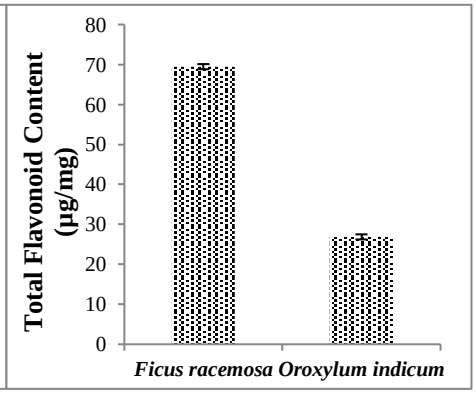

Figure 16.4: Total flavanoid content (µg/mg)

Figure.: Total Flavanoid Content (TFC)

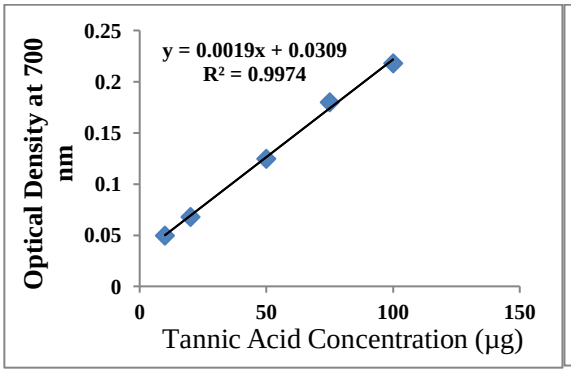

Figure 16.5: Standard graph for Tannic acid (µg/mg)

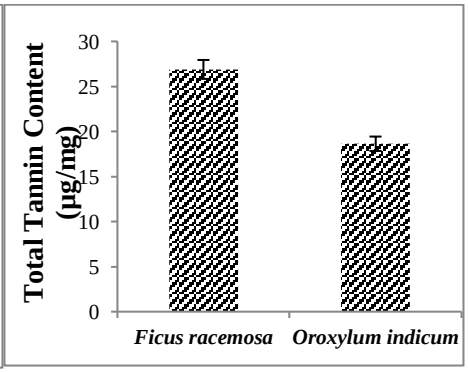

Figure16.6: Total tannin content

Figure: Total Tannin Content (TTC)

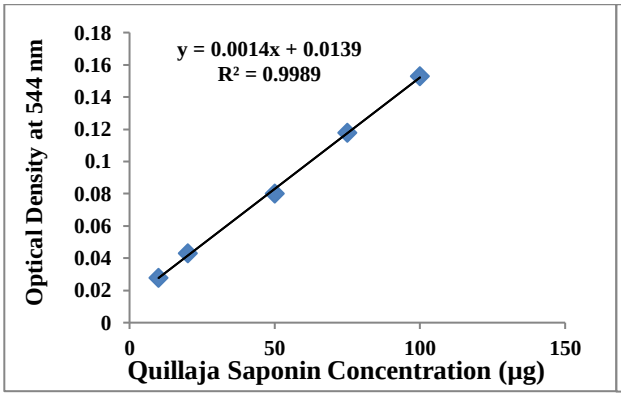

Figure 16.7: Standard graph for Quillaja Saponin

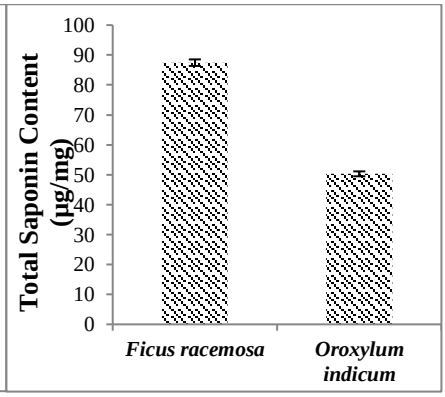

Figure 16.8: Total saponin content (µg/mg)

Figure: Total Saponin Content (TSC)

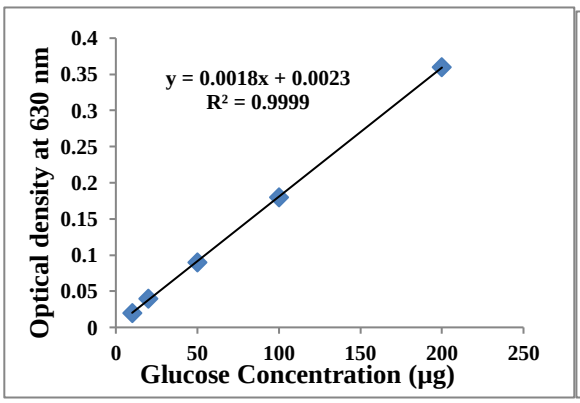

Figure 16.9: Standard graph for Glucose

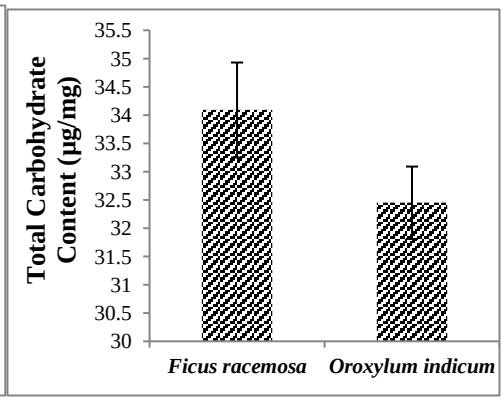

Figure 16.10: Total carbohydrate content (µg/mg)

Figure: Total Carbohydrate Content (TCC)

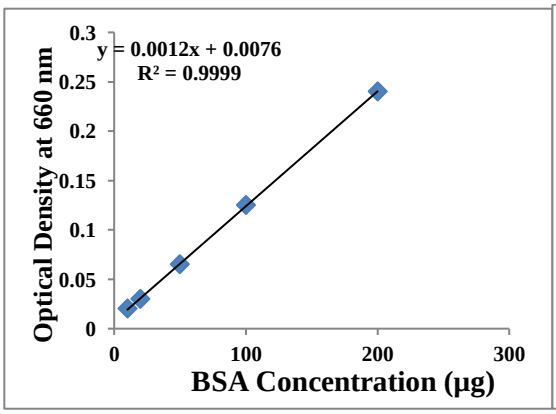

Figure 16.11: Standard graph for BSA

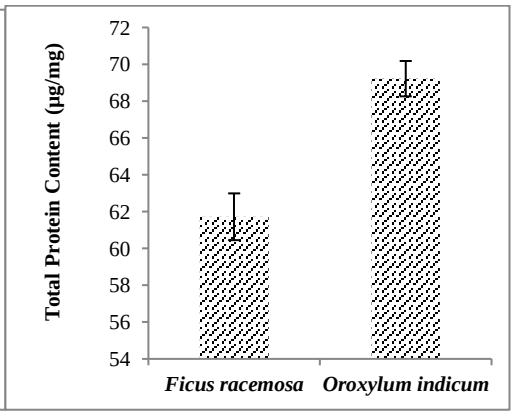

Figure. 16.12: Total protein content (µg/mg)

Figure: Total Protein Content (TPC)

Quantitative analysis of *F. racemosa* and *O. indicum* revealed rich composition of secondary metabolites in aqueous leaves extracts. Phytochemical composition of these plant species was reported earlier by various workers. Bhat *et al.,* 2019 and Dhas *et al.,* 2021 have reported presence of different phytochemical components viz; alkaloid, glycosides, saponins, tannins, flavanoids, terpenoids, reducing sugars, phytosterols, phenolic compounds, resins, proteins and amino acid in *F. racemosa* extracts prepared in different solvents (aqueous, chloroform, methanol and acetone). LC-MS analysis of this plant's aqueous leaf extract showed presence of phloretin, choline, citronellol, gallocatechin-4beta-ol, 1,11-undecanedicarboxylic acid, xi-p-menth-3-ene, gamma-nonalactone, adonirubin, delcosine and amitenone of which; phloretin (64.165%) and choline (11.933%) showed higher percentage area (Dhas *et al.,* 2021). The study indicates the antimicrobial potentiality of the plant leaves. However, studies related to total content of flavanoid, tannin, saponin and other components like protein and carbohydrate in *F. racemosa* leaves are very limited. Mohiuddin and Lia (2020) have found 49.44 mg of GAE/gm total phenolic content in aqueous fruit extract of *F. racemosa*. Earlier reports and current finding therefore indicate *F. racemosa* a good source of phenolic compounds. In the present study, leaf phenolic content of *F. racemosa* was calculated 68.20±0.27 µg/mg. The other phytochemicals viz; flavanoid, tannin, saponin was calculated 69.46±0.64 µg/mg, 26.89±1.05 µg/mg and 87.45±1.09 µg/mg respectively. From these observations it can be concluded that leaves of *F. racemosa* are potential source of many secondary metabolites. These secondary metabolites ultimately contribute to the health and nutrition of eri silkworms. On the other-hand, phytochemical analysis of *O. indicum* showed presence of phenols, flavanoids, tannins and saponins in an amount of 58.84±0.72 µg/mg, 26.87±0.64 µg/mg, 18.64±0.80 µg/mg and 50.30±0.82 µg/mg respectively. *O. indicum* is a potential source of secondary metabolites and an important part of traditional therapeutic processes. Almost all the parts of this plant are used in many health-related issues such as, diarrhea, dysentery, respiratory disorders, high blood pressure, jaundice etc. (Dinda *et al.,* 2015). Earlier researches quantified different phytochemical parameters of *O. indicum* leaves prepared in different solvents. These reports have shown solvent dependent composition of targeted phytochemicals. Asmaliyah*et al* (2016) performed an extensive analysis of flavanoid, steroid, tannin, saponin and alkaloid content in *O. indicum* collected from different places of Sumatra, Indonesia. Aqueous leaf extracts of this plant showed highest quantity of flavanoid (18.659%), steroid (12.228%), tannin (0.201%), alkaloid (26.027%) and saponin (0.154%) collected from Gandasuli, Kungkilan and Kampai area of Sumatra Island. Another study by Abdulhafiz *et al* (2022) emphasized the total phenolic, total flavanoid and LC-TOF-MS/MS analysis of *O. indicum*'s ethanolic and aqueous leaf extracts. Ethanolic leaf extract however showed higher phenolic (165 mg GAE/g) as well as flavanoid (101 mg Catechin euivalents/g) content as compared to aqueous leaf extract. Ethanolic extract also showed presence of compounds such as orientin, chrysin, pinoquercetin, cupressuflavone, puerarin, xyloside, forsythiaside and paederoside. Total phenolic and total flavanoid content was found 30 mg gallic acid equivalents/g and 76 mg catechin equivalents/g respectively in aqueous leaf extract. Both these data provided scientific validation regarding rich phytochemical composition and medicinal properties of *O. indicum*. The reports of the earlier studies can also be correlated with the present finding to interpret the phytochemical composition of *O. indicum*. However, in between these two plant species; in the present study, total contents of all the parameters were found more in *F. racemosa* as compared to *O. indicum*. Certain kind of variation is observed in between host plants used for different sericigenous insects (Changmai*et al.,* 2015; Chetia and Changmai, 2018; Thanga *et al.,*

2021). These variations finally determine superiority of one plant species over another and its preference as primary, secondary, tertiary or quaternary host plant for sericulture. Secondary metabolites have a determining role in this case. Secondary metabolites are ubiquitous in all plant organs and are therefore part of consumer's diet. Secondary metabolites such as flavanoids, phenolic compounds viz; caffeic acid, gallic acid and coumaric acid are antioxidants and can exert defense against pathogens and predators. Other secondary metabolites like tannins that initiate growth and impart protection to plants, improve feed efficiency and health of animals; thereby promotes growth and development (Patra and Saxena, 2011; Aboagye *et al.*, 2019). Patra and Saxena (2011) described the role of plant tannins as important nutrient factors in ruminant health. Shen *et al* (2021) explained probable impact of plant tannins in metabolism or gut micro biota in order to improve meat quality. Likewise, plant saponins facilitate significant growth, feed intake and absorption of foods in animals (Das *et al.,* 2012).

Role of these secondary metabolites in *S. ricini* is poorly understood and need proper attention and analysis. Protein and carbohydrate are other vital components necessary for growth and development of *S. ricini*. During the study, protein was recorded more (69.22±0.96 µg/mg) in *O. indicum* as compared to *F. racemosa* (61.72±1.27 µg/mg). Conversely, carbohydrate was recorded 34.09±0.84 µg/mg in *F. racemosa* and 32.45±0.64 µg/mg in *O. indicum*. For insect nutrition, proteins and amino acids are essential. These molecules promote growth and development, synthesizes enzymes and hormones needed for physiology and body metabolism, strengthen body muscles, build new tissues, maturation of ovaries and eggs, help in production of egg yolk (Hanife, 2006; Karasov and Douglas, 2013; Gall and Behmer, 2014). Similarly, carbohydrates act as source of energy (Hanife, 2006) and are building blocks for construction of chitin (Elieh-Ali-Komi and Hamblin, 2016). Certain types of carbohydrates and proteins also resist lethal effects of cold temperature by acting as antifreeze in insect's body fluid (Wen *et al.,* 2016). However, role of protein and carbohydrate in larval life of *S. ricini* still not described well.

The use of these two plant species within Kokrajhar district is restricted to some parts and within few erirearers only. The adoption of these two plant species in ericulture sector of Kokrajhar district was recorded for the first time. Earlier, use of *O. indicum* leaves for ericulture was recorded by Ahmed *et al* (2012) in Jorhat district of Assam. But use of *F. racemosa* as alternative plant species was not reported yet.

There were reports that revealed the utilization of *F. benghalensis* (Ahmed *et al*., 2012; Naik and Murthy, 2013) and *F. religiosa* (Naik and Murthy, 2013) as tertiary food plants for *S. ricini*. Thus, the current study as well as secondary literature clearly indicates the potentiality of Genus: Ficus (Family: Moraceae) as alternate host plant for eri silkworms. During the study, erirearers were found satisfied with the use of these two plant species. Leaves of these plants were mostly used during winter when *R. communis* shade leaves and less available in wild. For chawki rearing, rearers supply the tender leaves of *O. indicum* and *F. racemosa* by mixing with leaves of *R. communis*. For late-stage rearing also, bunch feeding of both the plants by mixing with *R. communis* were performed. This technique of fortifying alternate food plants with primary host plant was tactically adopted by the rearers with an aim to maintain generation, control winter crop loss and malnutrition of *S. ricini* due to food scarcity.

16.4 Conclusion:

Nutrition and health of *S. ricini* depends on the type and nutrient composition of host plants. The present study manifested the rich phytochemical composition of two newly adopted host plants of *S. ricini*, *O. indicum* and *F. racemosa* in some parts of Kokrajhar district. Adoption of these plants however is based on seasonal unavailability of primary host plant *R. communis*, but feeding preference, overall growth and development of *S. ricini* confirms their potentiality as alternative host plants in ericulture sector of Kokrajhar district. Therefore, further investigation regarding efficiency of *F. racemosa* and *O. indicum* as well as grainage performances of *S. ricini* reared on both the plants are necessary to figure out in order to authenticate the usefulness of these two plant species in ericulture.

16.5 Acknowledgement:

We are grateful to erirearers for sharing the information related to our study and helped us in collection of plant leaves for analysis. We are also thankful to Guwahati Biotech Park for helping us in preparation of extracts necessary for analysis.

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