

Secondary metabolite analysis of selected plants and antifeedant activity against tobacco cutworm

K K Abitkar, S S Khavare, S S Padalkar, P D Shiragave*

Department of Agrochemicals and Pest Management, Devchand College, Arjunagar, Maharashtra, India

Abstract

The present study aimed to investigate phytochemical composition and antifeedant potential of selected plant extracts found in and around Kolhapur district. Acetone, chloroform, methanol and aqueous extracts of *Strobilanthes callosa* Nees, *Cascabela thevetia* (L.) and *Terminalia paniculata* B. Heyne ex Roth were analyzed for total tannin, phenol and alkaloid content using standard spectrophotometric methods. Antifeedant activity was evaluated against *Spodoptera litura* using a no-choice bioassay method. The results revealed significant variations in phytochemical composition and content in plant extracts of *S. callosa*, *C. thevetia* and *T. paniculata*. Highest tannin content was recorded in methanolic extract of *T. paniculata* (0.624 ± 0.010 mg TAE/g DW) and phenol in methanolic extract of *S. callosa* (0.848 ± 0.013 mg CE/g DW). Alkaloid content was found to be highest in methanolic extract of *C. thevetia* (0.690 ± 0.043 mg QE/g DW). The antifeedant bioassay demonstrated that *C. thevetia* methanolic extract showed the strongest antifeedant activity ($58.99 \pm 0.066\%$) with a significant reduction in food consumption.

Keywords: Phytochemical, bioassay, *Spodoptera litura*, antifeedant activity, dry weight

Introduction

Agriculture production is limited by various factors including attack by insect pests in large scale. *Spodoptera litura* (Fab.) (tobacco cutworm) is a major agricultural pest that can seriously harm a wide range of crops. This polyphagous pest larvae cause serious damage by feeding on the leaves of numerous commercially significant plants including cotton, tobacco, vegetables, and ornamental plants. *S. litura* is broadly distributed throughout the east and south Asia, Oceania and some African islands and Hawaii within climate types ranging from tropical to temperate regions (Bhosale, 2019) [3]. Although chemical pesticides have greatly helped humanity in terms of agricultural production over the past couple of decades and have been contributing significantly to crop protection programs. Chemical pesticides pose a substantial threat to the environment, human health and animal health (Damalas and Eleftherohorinos, 2011; Sharma *et al.*, 2019) [5, 23].

In recent years there has been a growing interest in exploring natural sources for ecofriendly alternatives to synthetic pesticides (Chandler *et al.*, 2011) [4]. Plant derived secondary metabolites act as a promising solution as a natural antifeedants and insect deterrents. Antifeedants interrupt the feeding habit of pests, lowering crop damage without the harmful effects of conventional pesticides. Secondary metabolites are organic compounds which are found in plants that indirectly takes part in their growth and development or reproduction but play a critical role in ecological interactions, such as defense mechanisms against insect-pests, pathogens herbivores and other environmental stressors (Kulshreshtha and Saxena, 2019; Gorawade *et al.*, 2022) [9, 17]. The compounds terpenoids, phenolics, flavonoids and, alkaloids have gained notable attention due to their potential applications such as antifungal, antibacterial, antioxidant or insecticidal properties in agriculture and pharmaceuticals (Lengai *et al.*, 2020) [18]. In particular, role of secondary metabolites in pest management strategies has become an area of attraction, as chemical pesticides raise concerns over resistance development, environmental impact and human health

hazards (Mishra, 2015; SahiraBanu and Catherin, 2015) [10, 19, 22]. These compounds not only protect plants from herbivory but also act as a sustainable solution for pest management in agriculture (Gupta and Dikshit, 2010) [10].

The study focuses on the analysis of secondary metabolite in *Strobilanthes callosa* Nees, *Cascabela thevetia* (L.) and *Terminalia paniculata* B. Heyne ex Roth to evaluate their effective antifeedant activity against *S. litura*, to contribute to the development of sustainable plant based pest management approaches that reduce environmental risks while significantly controlling pest populations.

Materials and methods

Collection extraction of the plant material

On the basis of literature survey, *Strobilanthes callosa* Nees, *Cascabela thevetia* (L.) and *Terminalia paniculata* B. Heyne ex Roth were selected as study material. Fresh plant leaves were collected from the Kolhapur district. Fresh leaves were cleaned with tap water, allowed to dry in the shade and then ground to powder using a grinder. The powder was soaked for 24 hours in distilled water, methanol, acetone and chloroform. The extract was filtered through Buchner funnel and filtrate was stored at 4°C until use (Eneh *et al.*, 2016) [7].

Qualitative phytochemical analysis

The plant extracts were subjected to several phytochemical qualitative tests using the standard protocols outlined by Harborne (1973) [11], Trease and Evans (1989) [27] and Sofowara (1993) [25]. All plant extracts were diluted to mg/ml concentration and used for further studies.

Quantitative estimation

Total phenol content (TPC)

To estimate total phenol content, add 200 µl (mg/ml) sample to a test tube, followed by 1 ml of Folin Ciocalteu Phenol Reagent and 0.8 ml 7.5 % sodium carbonate after 5 minutes. Incubate the mixture for 60 minutes at room temperature. Measure the absorbance at 765 nm. Catechin was used as standard (Yadao *et al.*, 2015) [29].

Total tannin content (TTC)

Folin Ciocalteu Phenol Reagent test was carried out to measure total tannin content. Add 100 µl plant extract to 7.5 ml of distilled water. Mix 500 µl Folin Ciocalteu Phenol Reagent and 35% 1 ml sodium carbonate and diluted to 10 ml with distilled water. Shake the mixture thoroughly and allowed to settle for 30 minutes at room temperature. The absorbance was measured at 700 nm. Tannic acid was used as a standard (Kavitha, 2016) [16].

Total alkaloids content (TAC)

To measure the total alkaloid content of plant extracts, 100 µl plant samples were treated with 1 ml of bromocresol green solution and 1 ml of phosphate buffer at pH 4.7. Shake the liquid thoroughly and then add 2ml of chloroform. The absorbance was measured at 470 nm by UV double beam spectrophotometer (Shimadzu, UV-1900i) Quercetin (10-60 µg/ml) was used as standard (Tan, 2018) [26].

Rearing of test insect

The rearing of *S. litura* was conducted as per the procedure given by Arivoli and Tennyson (2013) [1]. Larvae of *S. litura* of various instars were collected from tobacco fields from Arjunnagar, District Kolhapur. The collected larvae of different instars were kept individually in a plastic container, packed with muslin cloth. The larvae were fed with castor leaves (*Ricinus communis* L.) under the laboratory conditions (27±1°C and 75±5% RH with natural photoperiod). Sterilized soil was provided for pupation. For the emergence of adults, pupae were collected from the soil and placed into the rearing cage. After the emergence of

adults, 10% honey solutions with few drops of multivitamins were feed to increase the rate of fecundity. Filter papers were used for laying egg and egg masses were allowed to hatch. The neonates (newly hatched larvae) were collected from the cage and feed with fresh castor (*Ricinus communis* L.) leaves. The whole rearing procedure was repeated and the culture was maintained throughout the study period.

Antifeedant activity

The antifeedant properties of several plant solvent extracts were assessed using leaf disc no choice methods described by Isman *et al.* (1990) [12] and Arivoli and Tennyson (2013) [1]. A fresh castor leaves (4 cm diameter disc) were prepared and dipped in 0.5 %, 1 %, 2.5% and 5% plant extracts. The treated discs were air dried and placed singly in a petridish (9 cm diameter). Pre-starved larvae of *S. litura* at third instar stage were placed in to each petridish. The larvae were allowed to ingest the treated castor leaf discs for 24 hours. Leaf discs treated with methanol, chloroform, acetone and water were used as positive and negative controls. The experiment was arranged in three replicates. After 24 hours, unconsumed leaf area was measured and calculated using the formula

$$\text{Percent (\%)} \text{ Antifeedant activity} = [(C-T) \div (C+T)] \times 100$$

Where, C- Leaf disc consumed by the larvae in control, T- Treated leaf disc consumed by the larvae.

Results and discussion

The plant extract extracted in methanol, acetone, chloroform and distilled water shows the presences of many secondary metabolites are recorded in Table 1.

Table 1: Qualitative phytochemical screening of leaf extracts of *S. callosa*, *C. thevetia* and *T. paniculata*.

Name of the plant	Solvents	Phenolics	Flavonoids	Tannins	Terpenoids	Alkaloids	Saponin
<i>Strobilanthes callosa</i>	Water	+	-	-	-	+	-
	Acetone	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+
	Chloroform	+	-	+	-	-	-
<i>Cascabela thevetia</i>	Water	+	-	+	-	+	+
	Acetone	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+
	Chloroform	+	-	-	+	-	+
<i>Terminalia paniculata</i>	Water	+	+	+	-	+	-
	Acetone	+	+	+	-	+	+
	Methanol	+	+	+	+	+	+
	Chloroform	+	-	+	-	-	-

“+” indicates chemical constituents is present “-” indicates chemical constituent is absent.

Qualitative phytochemical analysis of *S. callosa*, *C. thevetia* and *T. paniculata* leaf extract in acetone, methanol, chloroform and distilled water was done in which, tannins and phenols were present in all the methanolic samples depicted in Table 1. Flavonoids were positive in methanol and acetone of all three plants extracts, aqueous extract of *T. paniculata* was positive where, negative in *S. callosa* and *C. thevetia*. Chloroform extracts showed the negative results. Test for terpenoids and saponins of *S. callosa* and *T. paniculata* revealed positive and all four extracts were positive in *C. thevetia*. Test for alkaloids revealed positive results in aqueous, acetone and methanolic extracts and negative in chloroform extracts of all three plants. Presence of phytochemicals in plants may be attributed to their repellent and insecticidal properties (Kabar and Gichia, 2001) [15].

Estimations of secondary metabolites

Total phenol content (TPC)

Estimation of total phenol content (TPC) in plant extracts were done by FCR method. Occurrence of blue color assures the positive result. From Figure 1, it is evident that, highest total phenol content was recorded in methanolic plant extract followed by acetone and aqueous extracts. As compared to methanolic extract chloroform extract showed negligible presence of phenolic content. Chloroform extract of *T. paniculata* contain lowest phenolics, where as methanolic extract of *S. callosa* recorded the highest phenolic content (0.848±0.013 mg CE/g DW) followed by methanolic extract of *C. thevetia* (0.809±0.046 mg CE/g DW) and acetone extract of *S. callosa* extract (0.709±0.019 mg CE/g DW) shown in Figure 1. Higher phenolic content is attributed in biological activities (Johari and Khong, 2019) [14].

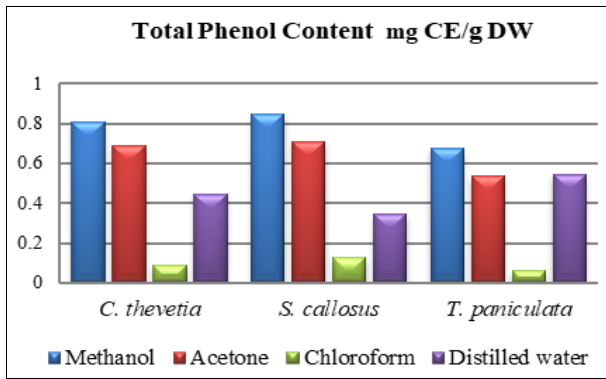


Fig 1: Total Phenol Content (mg CE/g DW)

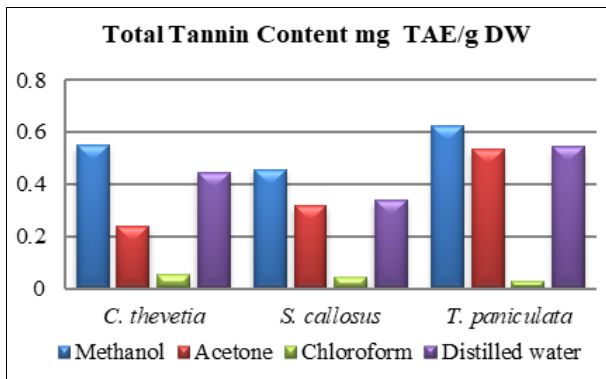


Fig 2: Total Tannin Content mg of *C. thevetia*, *S. callosa* and *T. paniculata*. TAE/g DW of *C. thevetia*, *S. callosa* and *T. paniculata*

Total tannin content (TTC)

FCR method was used for the estimation of the total tannin content in plant extracts. Figure 2. shows the graphical data of tannin estimation. Similar to phenolic, methanolic plant extract exhibited the presence of highest total tannin content, followed by acetone, aqueous and chloroform extracts. Chloroform extract of *T. paniculata* recorded the lowest tannin content (0.029±0.006 mg TAE/g DW) where

as methanolic extract of *T. paniculata* recorded the highest tannin content (0.624±0.010 mg TAE/g DW) followed by methanolic extracts of *C. thevetia* (0.550±0.026 mg TAE/g DW) and *S. callosa* (0.458±0.013 mg TAE/g DW) respectively. El-Aswad *et al.* (2023) [6] has reported significant antifeedant activity of tannin and growth inhibition action. Hence, tannins could be undoubtedly a new insect control agents required in pest management.

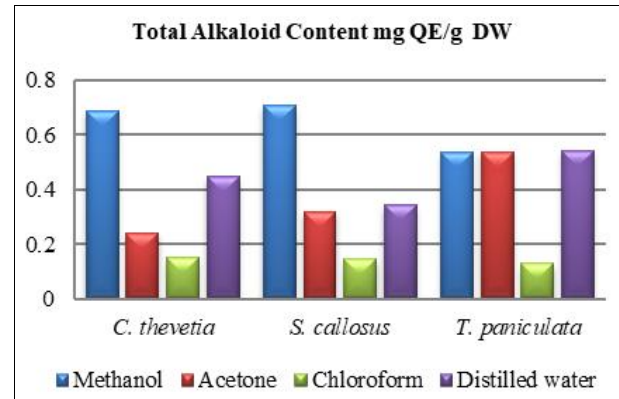


Fig 3: Total Alkaloid Content mg QE/g DW of *C. thevetia*, *S. callosa* and *T. paniculata*

Antifeedant activity

Alkaloids

Total alkaloid content is graphically presented in Figure 3. Methanolic plant extract exhibited presence of highest total alkaloid content followed by aqueous, acetone and chloroform extracts. Chloroform extracts exhibited lowest alkaloids content. Methanolic extract of *S. callosa* and *C. thevetia* showed highest alkaloid content 0.709±0.002 QE/g DW and 0.69±014 mg QE/g DW respectively.

Antifeedant activity of *C. thevetia*, *S. callosa* and *T. paniculata* leaf extract against *Spodoptera litura* is depicted in Table 2.

Table 2: Percentage Antifeedant activity of *C. thevetia*, *S. callosa*, *T. paniculata* leaf extract against *Spodoptera litura*.

Sr. No.	Solvent	Treatment (%)	Antifeedant Activity (%)		
			<i>S. callosa</i>	<i>C. thevetia</i>	<i>T. paniculata</i>
1.-	Acetone	0.5	12.84±0.08	9.15±0.008	6.28±0.002
		1.0	16.65±0.01	11.68±0.11	11.08±0.109
		2.5	30.00±0.19	18.32±0.015	15.96±0.23
		5.0	38.12±0.21	42.67±0.003	28.36±0.32
2.	Methanol	0.5	11.84±0.085	14.06±0.12	11.23±0.56
		1.0	21.36±0.023	25.32±12	17.23±0.003
		2.5	32.46±0.002	32.84±0.085	22.37±0.012
		5.0	45.78±0.012	58.99±0.066	31.29±0.208
3.	Water	0.5	-	9.32±0.65	-
		1.0	9.32±0.65	10.92±0.104	-
		2.5	14.52±0.37	19.32±0.32	10.32±0.006
		5.0	20.26±0.32	27.36±0.12	18.29±0.26
4.	Chloroform	0.5	-	11.68±0.1	-
		1.0	11.92±0.104	14.39±0.002	9.32±0.003
		2.5	17.66±0.177	19.32±0.023	12.35±0.023
		5.0	29.26±0.026	37.12±0.215	25.28±0.25
5.	Azadiractin	1.0	52.56±0.003	-	-
6.	Negative control	-	-	-	-

In addition, present study noted the dose dependant antifeedant activity in tested solvent extracts against the third instar larvae of *S. litura*. Methanolic extract of *C. thevetia* (5%) recorded the highest antifeedant activity

(58.99±0.066 %) followed by *S. callosa* (45.78±0.012 %). Aqueous extract of *T. paniculata* and *S. callosa* (0.5 %) recorded the lowest antifeedant activity Jayesankara (2014) has reported antifeedant activity of crude extract of

Bossiaea buxifolia in different concentrations against *S. litura* and *H. armigera*. Arora *et al.* (2016) [2] previously reported potential antifeedant activity of *Polyalthia longiafoila* methanol extract with least concentration (1.0%) against *S. litura*. According to Vetal and Pardeshi (2019) [28] the ethanol solvent extract of *Argemone mexicana* showed higher larvicidal property against third instars larvae of *S. litura*. Insecticidal potentiality of *Exacum pendunculatum* was revealed against *S. litura* by Shiragave (2020) [24], he has reported highest ovicidal and larvicidal activity in methanolic plant extract (50 mg/ml).

Bioefficacy of Garlic extract, Aak leaf extract and biopesticides was reported by Narayan *et al.* (2021) [20] on mustard aphid. Similarly Gorawade *et al.* (2021) [8] has reported the significant antifeedant activities in methanol extracts of *Chromolaena odorata* ($82.45 \pm 0.16\%$) and *Leonotis nepetifolia* ($71.77 \pm 0.73\%$) respectively, at 5.0% concentration against *S. litura*.

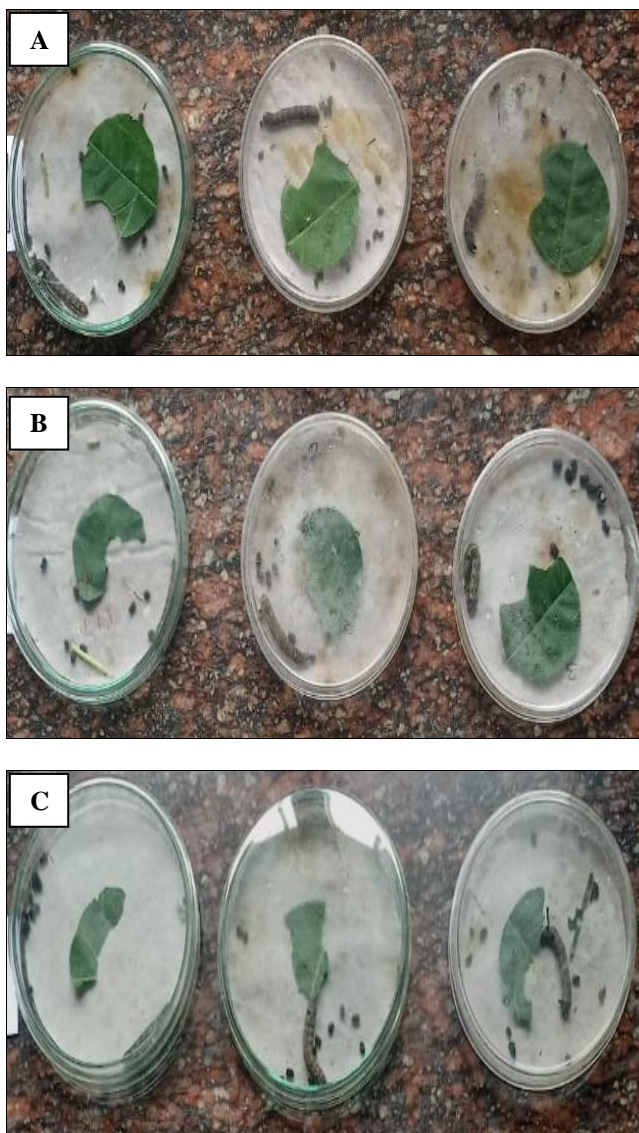


Fig 4: A. Highest antifeedant activity of Methanol extract of *C. thevetia* at 5 % concentration. B. Antifeedant activity at 5 % concentration of Methanol extract *S. callosa* extract. C. Antifeedant activity at 0.5 % concentration of Acetone extract of *T. paniculata* extract.

Jaysankara (2014) has reported antifeedant activity of crude extract of *Bossiaea buxifolia* in different

concentrations against *S. litura* and *H. armigera*. Arora *et al.* (2016) [2] previously reported potential antifeedant activity of *Polyalthia longiafoila* methanol extract with least concentration (1.0%) against *S. litura*. According to Vetal and Pardeshi (2019) [28] the ethanol solvent extract of *Argemone mexicana* showed higher larvicidal property against third instars larvae of *S. litura*. Insecticidal potentiality of *Exacum pendunculatum* was revealed against *S. litura* by Shiragave (2020) [24], He has reported highest ovicidal and larvicidal activity in methanolic plant extract (50 mg/ml). Bioefficacy of Garlic extract, Aak leaf extract and biopesticides was reported by Narayan *et al.* (2021) [20] on mustard aphid. Similarly Gorawade *et al.* (2021) [8] has reported the significant antifeedant activities in methanol extracts of *Chromolaena odorata* and *Leonotis nepetifolia* ($82.45 \pm 0.16\%$ and $71.77 \pm 0.73\%$) respectively, at 5.0% concentration against *S. litura*.

Conclusion

The study investigated that, *S. callosa*, *C. thevetia* and *T. paniculata* are rich in phytochemicals such as phenols, tannins, alkaloids, flavonoids, terpenoids and saponins. Methanolic extract of *S. callosa* recorded the highest phenolics and alkaloid content. Methanolic extract of *T. paniculata* recorded the highest tannin content. Methanolic extracts of *S. callosa*, *C. thevetia* and *T. paniculata* are exhibited maximum deterrence to tobacco cutworm. The plants selected for present study would be a potential source as phytopesticides against tobacco cutworms.

Acknowledgement

We are thankful to DST-FIST, Science Instrumentation Facility Centre (SIFC), Devchand College, Arjunagar for providing laboratory facility.

Conflict of interest

There is no conflict of interest involved in the study

References

1. Arivoli S, Tannyson S. Antifeedant activity, developmental indices and morphogenetic variations of plant extracts against *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae). Journal of Entomology and Zoology Studies, 2013;1(4):87-96.
2. Arora S, Mogha N, Bhardwaj T, Srivastava T. Antifeedant and Insecticidal Activity of Plant Extracts Against *Spodoptera litura* (Fab.) and *Lipaphis erysimi*. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 2016.
3. Bhosale AM. Bioefficacy of plant extracts against *Spodoptera litura* fab. (Lepidoptera: Noctuidae). International Journal of Entomology Research, 2019;4(3):48-52.
4. Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP. The development, regulation and use of biopesticides for integrated pest management. Philosophical Transaction of The Royal Society B, 2011;366:1987-1998.
5. Damalas C, Eleftherohorinos I. Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. International Journal of Environmental Research and Public Health, 2011;8:1402-1419.
6. El-Aswad AF, Aisu1 J, Khalifa MH. Biological activity of tannins extracts from processed *Camellia sinensis*

- (black and green tea), *Vicia faba* and *Urtica dioica* and *Allium cepa* essential oil on three economic insects. *Journal of Plant Diseases and Protection*,2023:130:495–508.
7. Eneh FU, Nwankwo EN, Okwuenu P. Phytochemical analysis and toxicity of *Casuarina equisetifolia* (whistling pine) to *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *International Journal of Entomology Research*,2016:1(3):10-17.
 8. Gorawade VB, Attar UA, Shiragave PD. Insecticidal potential and thin layer chromatographic profiling of *Chromolaena odorata* L. and *Leonotis nepetifolia* (L) R.Br. leaf extracts against *Helicoverpa armigera* (Hubner). *International Journal of Entomology Research*,2021:6(2):46-51.
 9. Gorawade V, Attar U, Shiragave P. Bioefficacy and GC-MS analysis of *Chromolaena odorata* and *Leonotis nepetifolia* leaf extracts against *Spodoptera litura*. *Journal of Crop Protection*,2022:11(3):361-375.
 10. Gupta S, Dikshit A. Biopesticides: An ecofriendly approach for pest control. *Journal of Biopesticides*,2010:3(1):186–188.
 11. Harborne JB. *Phytochemical methods*, London. Chapman and Hall,1973:49:188.
 12. Isman MB, Koul O, Luczynski A, Kaminski J. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *Journal of Agriculture and Food Chemistry*, 1990, 38(6).
 13. Jeyasankar A, Chinnamani T, Chennaiyan V, Ramar G, Arignar A. Antifeedant Activity of *Barleria buxifolia* (Linn.) (Acanthaceae) Against *Spodoptera litura* Fabricius And *Helicoverpa armigera* Hübner (Lepidotera:Noctuidae). *International Journal of Natural Sciences Research*,2014:2(5):78-84.
 14. Johari MA, Khong HA. Total Phenolic Content and Antioxidant and Antibacterial Activities of *Pereskia bleo*. *Advances in Pharmacological Sciences*, 2019, 1-4.
 15. Kabar JM, Gichia L. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against three arthropods. *African journal of Science and Technology*,2001:2(2):44-49.
 16. Kavitha CI, Indira G. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of *Strobilanthes kunthiana* (Neelakurinji). *Journal of Medicinal Plants Studies*,2016:4(4):282-286.
 17. Kulshreshtha A, Saxena J. Alkaloids and Non Alkaloids of *Tabernaemontana divaricata*. *International Journal of Research & Review*,2019:6(8):517-524.
 18. Lengai GMW, Muthomi JW, Mbega ER. Phytochemical activity and role of botanical pesticides in pest management of sustainable agriculture crop protection. *Scientific African*,2020:7(2020):00239.
 19. Mishra M, Gupta K, Kumar S. Impact of the Stem Extract of *Thevetia nerifolia* on the Feeding Potential and Histological Architecture of the Midgut Epithelial Tissue of Early Fourth Instars of *Helicoverpa armigera* Hübner. *International Journal of Insect Science*,2015:7:53-60.
 20. Narayan K, Tomar RKS, Awasthi AS, Pandey TD, Chaure NK, Salame L. Bioefficacy of different biopesticides and botanicals against mustard aphid (*Lipaphis Erysimi* Kalt.). *International Journal of Entomology Research*,2021:6(1):72-74.
 21. Sahira Banu K, Cathrine L. General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science (IJARCS)*,2015:2(4):25-32.
 22. Sedek MM. Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Applied Entomology*,2003:27:396-404.
 23. Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu G, Handa N, et al. Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*,2019:1:1446.
 24. Shiragave PD. Phytochemical profiling, ovicidal, larvicidal potential of *Exacum pendunculatum* L. against *Spodoptera litura*. *International Journal of Entomology Research*,2020:5(6):234-238.
 25. Sofowara A. *Medicinal plants and Traditional medicine in Africa*. Spectrum Books Ltd Ibadan, Nigeria, 1993, 191-289.
 26. Tan PV. The Determination of Total Alkaloid, Polyphenol, Flavonoid and Saponin Contents of Pogangan (*Curcuma sp.*). *International Journal of Biology*, 2018, 10(4).
 27. Trease GE, Evans WC. *Pharmacognsy*. 11th edn. Brailliar Tiridel Can. Macmillian publishers, 1989.
 28. Vetral DS, Pardeshi AB. Larvicidal potential of *Argemone mexicana* L. Plant extracts against *Spodoptera litura* fab. *The Pharma Innovation Journal*,2019:8(6):698-702.
 29. Yadao N, Priya CL, Bhaskara Rao KV. Carbohydrate hydrolyzing enzyme inhibitor property, antioxidant and phytochemical analysis of *Cassia auriculata*, *Delonix regia* and *Vinca rosea* Linn: an *in vitro* study. *Journal of Applied Pharmaceutical Science*,2015:5(05):018-027