

Effect of *Chromolaena odorata* extracts on antioxidant defence system of *Odoiporus longicollis*

Drishyaraj KP

Assistant Professor, Department of Chemistry, Regional College of Science and Humanities, Kizhisseri Malappuram, Kerala, India

Abstract

Odoiporus longicollis, a major monophagous pest of banana, poses a significant threat to plantations in southern India, particularly Kerala. Current control methods rely on chemical insecticides, necessitating eco-friendly alternatives. This study investigates the potential of *Chromolaena odorata* leaf extract for weevil management. Toxicity bioassays were conducted to assess its effects on *O. longicollis*, alongside enzyme assays measuring detoxifying enzymes and biochemical profile changes. The extract, prepared using an acetone-based soaking method, was tested in varying concentrations, determining the median lethal dose (LD₅₀) and sub-lethal effects over 24–96 hours. Results highlight the potential of *C. odorata* as a natural pest control agent, reducing reliance on chemical insecticides.

Keywords: *Chromolaena odorata*, *Odoiporus longicollis*, antioxidant defense system

Introduction

Pest insects significantly impact agriculture, the environment, and human health by damaging crops and reducing yields. Around 10% of global agricultural production is lost to pests before harvest. Integrated pest management strategies often include plant-derived botanical pesticides, such as extracts and essential oils, which offer eco-friendly alternatives to synthetic chemicals that cause resistance and ecological harm (Georghiou & Taylor, 1986; Isman & Akhtar, 2007).

This study evaluates the toxicity of *Chromolaena odorata* leaf extract on *Odoiporus longicollis*, a major banana pest in southern India. It aims to assess toxicity bioassays, enzymatic detoxification responses, and biochemical changes in the pest. The hypothesis is that *C. odorata* extract will exhibit toxicity, altering enzyme activity and biochemical profiles, providing a sustainable pest control option.

Phytochemicals

Medicinal plants play a crucial role in healthcare due to their bioactive compounds (Edeoga *et al.*, 2005). Though not essential nutrients, phytochemicals like alkaloids, tannins, flavonoids, and phenolics contribute to health benefits (Srivastava *et al.*, 2011; Hill, 1952). Over 2000 phytochemicals have been identified, with plant-based diets linked to reduced risks of cancer and cardiovascular diseases (Taiz & Zeiger, 2006; Shui & Lai Pang, 2004).

Flavonoids, found in food plants and traditional medicines, possess pharmacological significance (Gurib-Fakim, 2006). *Abelmoschus esculentus* (okra) is valued for its nutritional and therapeutic properties, including antioxidant and anti-diabetic effects (Middleton *et al.*, 2005; Saha *et al.*, 2011). *Chromolaena odorata*, despite being invasive, has medicinal applications such as anti-inflammatory and anti-hypertensive properties (Mbagwu *et al.*, 2010).



Chromolaena odorata



Odoiporus longicollis

Chromolaena odorata is widely used for wound healing, with fresh leaves or extracts applied to burns and skin infections. In Vietnam, its aqueous extract is licensed as Eupolin for clinical use (Vu *et al.*, 2007). Studies show it promotes wound healing by enhancing adhesion complexes and fibronectin in keratinocytes (Kang *et al.*, 2013) while its antimicrobial properties aid recovery (Bui *et al.*, 2008).

Research confirms its analgesic, anti-inflammatory, and antipyretic effects (Orisakwe *et al.*, 2006; Akinmoladun *et al.*, 2016; Chukwujekwu *et al.*, 2007). Its medicinal potential is linked to phenolic acids and flavonoids, contributing to strong antioxidant activity and tissue repair (Abdullahi *et al.*, 2012; Yao *et al.*, 2009).

Odoiporus longicollis (Banana stem weevil)

The banana stem weevil (*Odoiporus longicollis*) is a major pest in banana and plantain cultivation across South and Southeast Asia, including India (Valmayor *et al.*, 1994)^[23]. Adults, usually black and 23–39 mm long, can also appear red in certain regions, a variation unrelated to sex (Dutt & Maiti, 1972)^[18]. Primarily nocturnal, they may fly during cooler or cloudy days.

With a lifespan of up to a year, the sex ratio is slightly female-biased (1:1.17) (Dutt & Maiti, 1972)^[18]. The pre-oviposition period lasts 15–30 days, and females lay about nine eggs daily into the pseudostem leaf sheath (Ranjith & Lalitha, 2001)^[22].



The larvae hatch in 3–8 days, appearing yellowish and fleshy. They tunnel into the pseudostem, feeding on succulent tissues and causing severe damage, especially in the pre-flowering stage. Tunneling can extend to the true stem, damaging flower buds and peduncles, leading to flower nonemergence and premature bunch decay (Padmanaban *et al.*, 2001)^[21]. In severe infestations, over 20% of plants may fail to flower.

Early infestations (~5 months) cause the most damage, with tunnels reaching up to 10 cm deep, weakening plants and increasing wind damage, especially in nematode-affected areas. Management strategies include clean planting materials, traps, and good agronomic practices (Gold *et al.*, 2001;

Masanza *et al.*, 2005). Insecticides have limitations due to resistance, cost, and environmental impact (Collins *et al.*, 1991; Gold *et al.*, 1999)^[28]. Biological control has been largely ineffective (Koppenhoffer *et al.*, 1992; Koppenhoffer & Schmutterer, 1993). Severe infestations can reduce yields by up to 90%.

Materials and Methods

This study examines the impact of *Chromolaena odorata* leaf extract on *Odoiporus longicollis*, a major banana pest.

Insects and plant samples were collected from Malappuram, Kerala. A sub-lethal extract concentration was tested over time, and biochemical changes were analyzed to assess its potential as a pest control strategy.

Collections of insects and plants

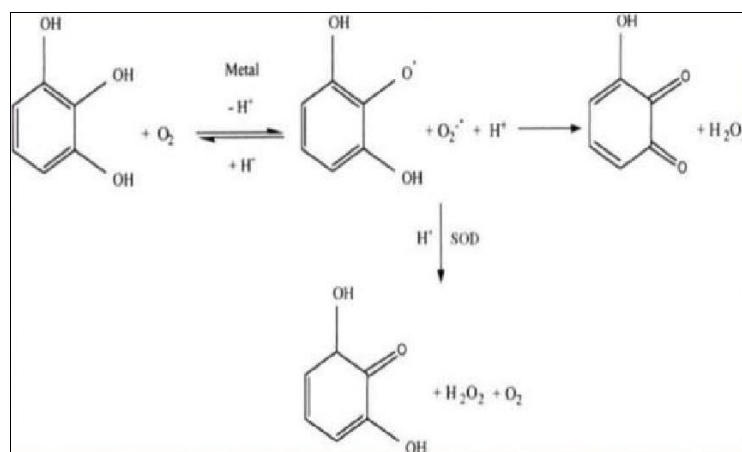
Adult *Odoiporus longicollis* insects were collected from the Malappuram district, Kerala, India, and were maintained in insect rearing cages at room temperature with banana stem as the diet. The leaves of *Chromolaena odorata* were collected from the same district, hand-picked, and thoroughly washed under running tap water before use.

The larvae hatch in 3–8 days, appearing yellowish and fleshy. They tunnel into the pseudostem, feeding on succulent tissues and causing severe damage, especially in the pre-flowering stage. Tunneling can extend to the true stem, damaging flower buds and peduncles, leading to flower nonemergence and premature bunch decay (Padmanaban *et al.*, 2001)^[21]. In severe infestations, over 20% of plants may fail to flower.

Early infestations (~5 months) cause the most damage, with tunnels reaching up to 10 cm deep, weakening plants and increasing wind damage, especially in nematode-affected areas. Management strategies include clean planting materials, traps, and good agronomic practices (Gold *et al.*, 2001; Masanza *et al.*, 2005). Insecticides have limitations due to resistance, cost, and environmental impact (Collins *et al.*, 1991; Gold *et al.*, 1999)^[28]. Biological control has been largely ineffective (Koppenhoffer *et al.*, 1992; Koppenhoffer & Schmutterer, 1993). Severe infestations can reduce yields by up to 90%.

Experimental Design

Experimental design, a sub-lethal concentration (one-tenth of the LD50 value, 10 mg) of *Chromolaena odorata* leaf extract was used to expose insects for 24, 48, 72, and 96 hours, with a control group remaining untreated. Each group contained 20 insects, which were homogenized, centrifuged, and the supernatant was analyzed for enzyme activity. Total protein was measured using Lowry's method, carbohydrates using the Anthrone method, and cholesterol using Zak's method. Enzyme activities, including peroxidase (POD), glutathione reductase (GR), and acetylcholinesterase (AChE), were determined by monitoring absorbance changes at specific wavelengths (420 nm, 340 nm, and 412 nm, respectively), and results were quantified based on standard curves.



SOD Activity

SOD activity is assessed by inhibiting pyrogallol autoxidation, with the reaction mixture containing the sample, Tris buffer, and pyrogallol. Absorbance at 420 nm is measured before and after one minute to determine the percentage inhibition of pyrogallol oxidation, reflecting the SOD activity. The test and control mixtures differ only in the presence of the sample. A unit of enzyme activity is defined as the enzyme that catalyzes the formation of 1 μmol of S-2,4-dinitrophenylglutathione per minute. Carboxylesterase activity is measured by hydrolyzing α -naphthyl acetate, with absorbance at 590 nm used to quantify the product. Other compounds like alkaloids,

flavonoids, glycosides, tannins, and polyphenols are estimated using colorimetric methods, with results expressed relative to specific standards.

Results and Discussion

Biochemical assay

The mortality bioassay determined an LD₅₀ of 0.5 mg/ml for *Chromolaena odorata* leaf extract. Table 1 shows significant biochemical changes in *Odoiporus longicollis*, with protein and carbohydrate levels gradually decreasing, while lipid levels initially rise before declining, indicating metabolic disruption.

Table 1: Effect of *Chromolaena odorata* Leaf Extract on Biomolecule Concentrations in *Odoiporus longicollis*

Biomolecules	Control	24 hours	48 hours	72 hours	96 hours
Protein	0.372	0.357	0.352	0.283	0.111
Carbohydrate	0.457	0.401	0.387	0.237	0.108
Lipid	0.023	0.071	0.012	0.011	0.007

Table 1 shows exposure to *Chromolaena odorata* extract caused a steady decline in protein (0.372 to 0.111) and carbohydrate (0.457 to 0.108) levels over 96 hours, indicating metabolic stress. Lipid levels spiked at 24 hours

(0.023 to 0.071) before dropping sharply (0.007 at 96 hours), suggesting lipid peroxidation. These changes highlight oxidative damage and potential toxicity to *Odoiporus longicollis*.

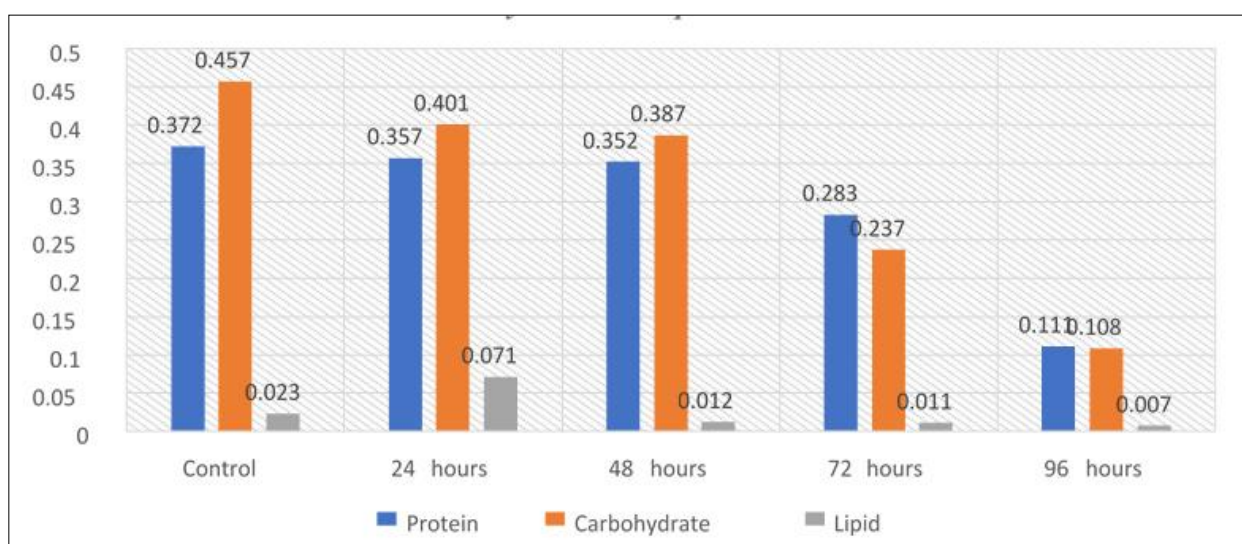


Fig 1: Changes in the levels of biomolecules protein, carbohydrate and lipid

Enzyme assay

Enzyme assay were done by seven detoxifying enzymes, Glutathione-S-transferases (GST), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), carboxylesterase (CAR), Glutathione reductase,

Acetylcholinesterase. Three of these enzyme levels were increasing first then become decreased. The increasing level indicates the resistance mechanism of *Odoiporus longicollis*. It reveals the significance of detoxification mechanism in *O. longicollis*.

Table 2: Activity Levels of GST, SOD, Acetylcholinesterase, and Glutathione Reductase at Different Time Intervals

Enzymes	Control	24 hours	48 hours	72 hours	96 hours
GST	0.593	0.975	1.216	0.391	0.384
SOD	2.54	2.425	1.35	1.29	0.206
Acetylcholinesterase	0.00178	0.00103	0.00084	0.00083	0.00082
Glutathione reductase	1.820	1.264	0.961	0.846	0.643

Table 2 shows that GST activity increased from 0.593 to 1.216 at 48 hours, then dropped sharply to 0.384 at 96 hours, indicating an initial detoxification response followed by enzyme depletion. SOD activity declined steadily from 2.54 to 0.206, suggesting oxidative stress.

Acetylcholinesterase activity decreased from 0.00178 to 0.00082, indicating possible neurotoxicity. Glutathione reductase levels dropped from 1.820 to 0.643, reflecting reduced antioxidant defense.

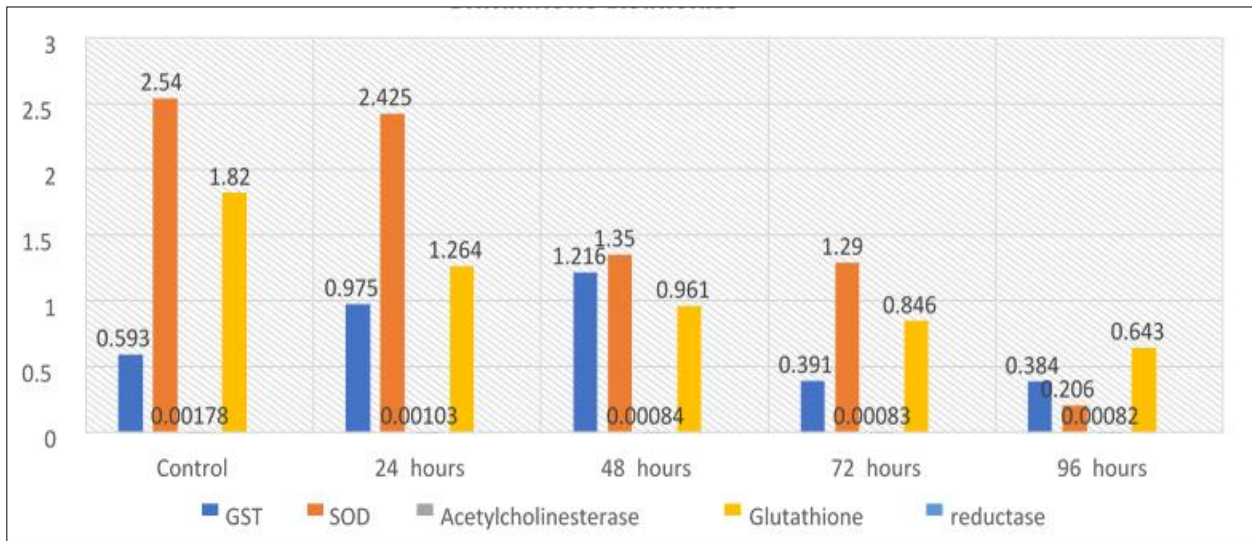


Fig 2: Activity Levels of GST, SOD, Acetylcholinesterase, and Glutathione Reductase at Different

Time Intervals

Table 3: Effect of *Chromolaena odorata* Leaf Extract on Peroxidase and Carboxylesterase Activity in *Odoiporus longicollis*

Enzymes	Control	24 hours	48 hours	72 hours	96 hours
Peroxidase	20.94	36.14	74.32	20.72	20.37
Carboxylesterase	270	250	230	190	180

Table 3 shows that peroxidase activity increased from 20.94 to 74.32 at 48 hours, then decreased slightly to 20.37 at 96 hours, suggesting an initial response to oxidative stress followed by a return to baseline levels. Carboxylesterase activity declined steadily from 270 to 180, indicating a

decrease in metabolic or detoxification capacity over time. These changes suggest *Chromolaena odorata* extract induces oxidative stress, with fluctuating enzyme responses in *Odoiporus longicollis*.

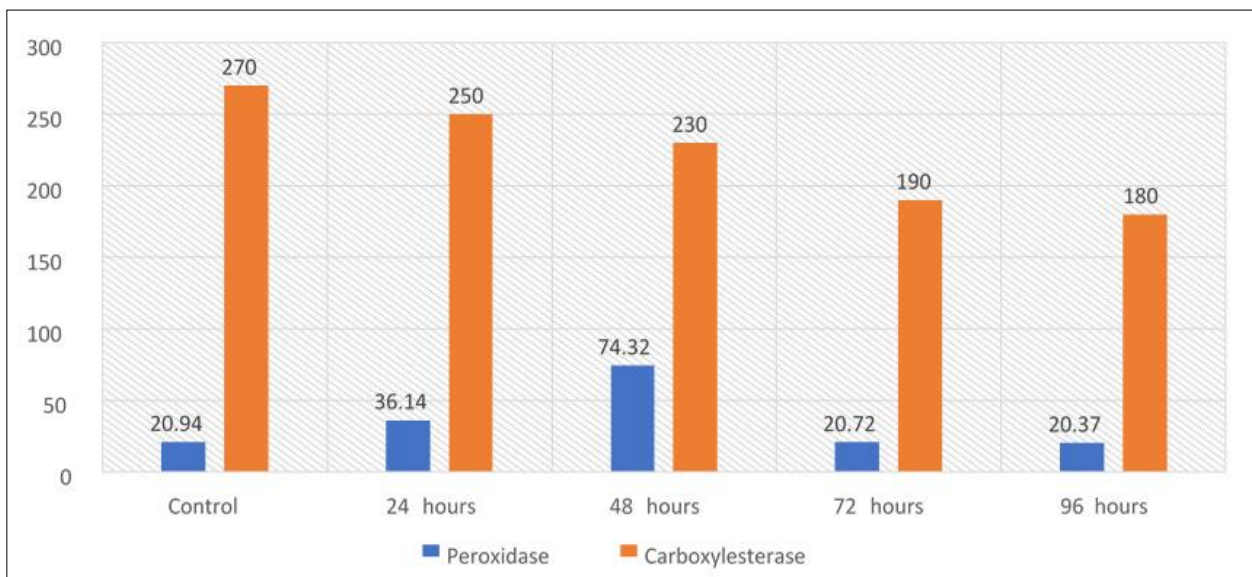


Fig 3: *Chromolaena odorata* Leaf Extract on Peroxidase and Carboxylesterase Activity in *Odoiporus longicollis*

Table 4: Effect of *Chromolaena odorata* Leaf Extract on Catalase Activity in *Odoiporus longicollis* Over Tim

Enzymes	Control	24 hours	48 hours	72 hours	96 hours
Catalase	14800	17020	18600	9760	31

Table 4 shows that catalase activity increased from 14,800 to 18,600 at 48 hours, indicating an initial oxidative stress response. However, activity dropped significantly to 31 by 96 hours, suggesting severe enzyme inhibition or depletion

due to prolonged exposure to *Chromolaena odorata* leaf extract. This sharp decline highlights potential oxidative damage and impaired antioxidant defense in *Odoiporus longicollis*.

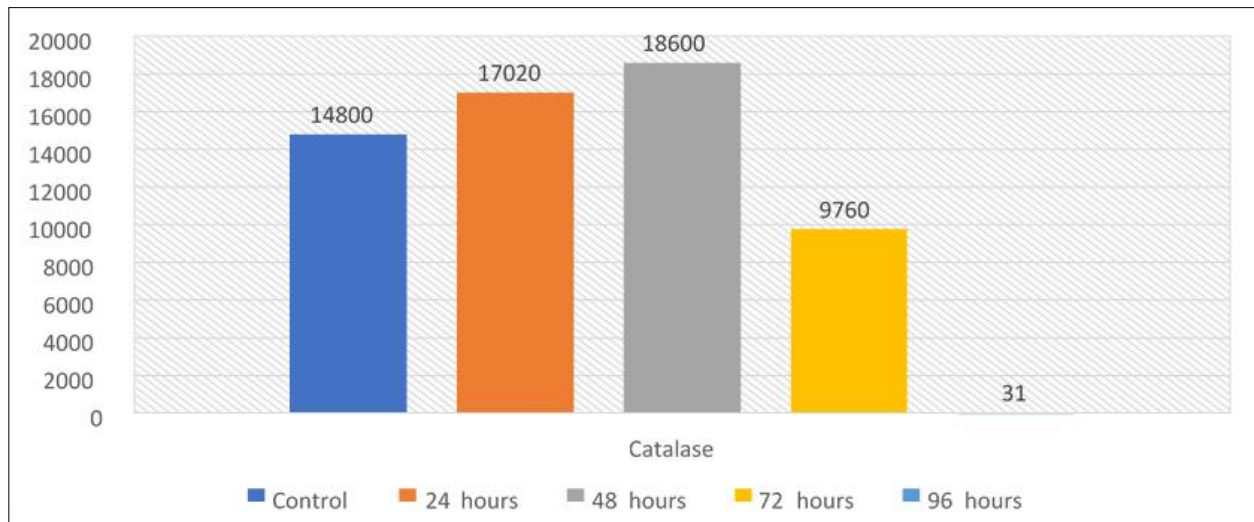


Fig 4: *Chromolaena odorata* Leaf Extract on Catalase Activity in *Odoiporus longicollis* Over Time

The phytochemical evaluation

The phytochemical evaluation of *Chromolaena odorata* leaf extract reveals notable levels of tannins (1.039 mg/g), phenols (0.903 mg/g), flavonoids (6%), alkaloids (0.884%), and glycosides (0.680%), indicating its strong antioxidant potential and possible therapeutic benefits. These compounds contribute to the extract's bioactivity, supporting its potential use in various health applications.

The study on the effect of *Chromolaena odorata* leaf extract phytochemicals on the detoxifying enzyme levels and biochemical aspects of the Banana stem weevil, *Odoiporus longicollis*, demonstrates the effectiveness of these phytochemicals in controlling the insect. The biochemical assays showed that the treatment with the leaf extract resulted in decreased concentrations of protein, lipid, and carbohydrate. Enzyme activity assays revealed an initial increase in detoxifying enzymes like catalase, peroxidase, Glutathione-S-transferase, and Acetylcholinesterase, which later decreased, indicating a resistance mechanism of the insect.

These findings align with previous research on allelopathy and its role in pest management, highlighting the potential of allelopathic compounds as natural alternatives to synthetic pesticides. The results suggest that phytochemicals from *C. odorata* could be effective in controlling *O. longicollis* populations in plantations without the environmental concerns associated with synthetic chemicals.

Conclusion

The study demonstrated the toxic effects of *Chromolaena odorata* leaf extract on *Odoiporus longicollis*, as indicated by significant biochemical and enzymatic changes. The mortality bioassay determined an LD₅₀ of 0.5 mg/ml, confirming the extract's potency.

Biochemical analysis revealed a progressive decline in protein and carbohydrate levels, while lipid levels initially increased before sharply decreasing, suggesting metabolic disruption and oxidative stress. Enzyme assays showed fluctuating detoxification responses, with initial increases in GST, peroxidase, and catalase activities followed by a sharp decline, indicating enzyme depletion and impaired antioxidant defence. The reduction in acetylcholinesterase and carboxylesterase activity suggests possible neurotoxic effects.

The phytochemical analysis identified tannins, phenols, flavonoids, alkaloids, and glycosides, which contribute to the extract's strong antioxidant potential. These findings highlight the bioactivity of *Chromolaena odorata*, suggesting its potential use in pest management due to its toxicity against *Odoiporus longicollis*. However, further studies are required to assess its environmental impact and long-term effects on non-target organisms.

References

- Balbaa SI, Sayed HH, Ashgan YZ. Medicinal plant constituents. 3rd ed. General Organization for University and School Books, 1981, 190-255.
- Cameron GR, Mitton RF, Allan JW. Measurement of flavonoids in plant samples. *Lancet*, 1943, 179.
- David M, Richard JS. In: Bergmeyer J, Grab M, editors. *Methods of enzymatic analysis*. Verlag Chemie Weinheim Deer Field Beach, Florida, 1983, 358.
- Finney DJ. *Probit analysis*. 3rd ed. Cambridge University Press, 1971, 333.
- Habig WH, Pabst MJ, Jokoby WB. Glutathione transferase: A first enzymatic step in mercapturic acid III formation. *J Biol Chem*, 1974;249:7130-7139.
- Harborne JB. *Phytochemical methods*. Chapman and Hall, 1973, 49-188.
- Hodge JE, Hofreiter BT. In: Whistler JN, Miller RL, Miller BE, editors. *Methods in carbohydrate chemistry*. Vol. 17. Academic Press, 1962, 420.
- Kranthi KR. *Insecticide resistance monitoring, mechanisms, and management manual*. Central Institute for Cotton Research, 2005, 78-82.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagent. *J Biol Chem*, 1951;193:265-275.
- Luck H. Catalase. In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. Academic Press, 1963 895-897.
- Mallick CP, Singh MB. *Plant enzymology and plant histoenzymology*. Kalyani Publishers, 1980, 286.
- Marklund S, Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*, 1974;47:469-474.
- Polshettiwar SA, Ganjiwale RO. Spectrophotometric estimation of total tannin in some Ayurvedic eye drops. *Indian J Pharm Sci*. 2007;69(4):574-576.

14. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effect of light and benzyladenine on peroxidase activity. *Cell Physiol*,1995:26:984.
15. Sadhasivam S, Manikam A. Biochemical methods for agricultural science. Wiley Eastern Limited, 1992.
16. Zak B, Dickenman RC, White EG, Burnett H, Cherdey PJ. Rapid estimation of free and total cholesterol. *Am J Clin Pathol*,1954:24:1307-1315.
17. Charles JSK, Thomas MJ, Menon R, Premalatha T, Pillai SJ. Field susceptibility of banana to pseudostem borer *Odoiporus longicollis* Oliver. In: Symposium on Technical Advancement in Banana/Plantain Production and Processing – India-International: Kerala Agricultural University, Mannuthy, India, 1996, 20-24.
18. Dutt N, Maiti BB. Bionomics of the banana pseudostem weevil, *Odoiporus longicollis* Oliv. (Coleoptera: Curculionidae). *Indian J Entomol*,1972:34:20-30.
19. Nahif AA, Padmanaban B, Sundararaju P. Ultrastructure of the banana pseudostem weevil, *Odoiporus longicollis* (Coleoptera: Curculionidae). In: Entomocongress 2000: Perspectives for the New Millennium: University of Kerala: Nov 5-8. Trivandrum, India, 2000 5-8.
20. Padmanaban B, Kandaswamy M, Uma S, Sathiamoorthy S. Relative susceptibility of *Musa* germplasm to banana stem weevil, *O. longicollis* Oliver (Coleoptera: Curculionidae). Unpublished.
21. Padmanaban B, Sundararaju P, Sathiamoorthy S. Incidence of banana pseudostem borer, *O. longicollis* Oliv. (Coleoptera: Curculionidae) in banana peduncle. *Indian J Entomol*, 2001, 63(2).
22. Ranjith AM, Lalitha N. Epideictic compounds from the banana pseudostem weevil, *O. longicollis* Oliver. In: Narasimhan S, Suresh G, Wesley SD, editors. Innovative pest and disease management in horticultural and plantation crops. SPIC Science Foundation, 2001, 59-61.
23. Valmayor RV, Davide RG, Staton JM, Treverrow NL, Roa VN, editors. Banana nematodes and weevil borers affecting bananas in Asia and the Pacific. INIBAP/ASPNET, 1994, 18-22.
24. Batista FA, Leite LG, Raga A, Sato ME. Enhanced activity of *Beauveria bassiana* (Bals.) Vuill. associated with mineral oil against *Cosmopolites sordidus* (Germar) adults. *Anais Soc Entomol Brasil*,1995:23(2):405-408.
25. Biaggioni RL, Micherfe MF, Silvana MT, Manuel PO, Janeiro N, Lema EL, Fancell M, Padilha J. Virulence and horizontal transmission of selected Brazilian strains of *Beauveria bassiana* against *Cosmopolites sordidus* under laboratory conditions. *Bull Insectol*,2011:64(2):201-208.
26. Brenes S, Carballo VM. Evaluación de *Beauveria bassiana* (Bals.) para el control biológico del picudo del plátano *Cosmopolites sordidus* (Germar). *Manejo Integrado de Plagas*,1994:31:17-21.
27. Castrillón C, Urrea CF, Cardona JE, Zuluaga LE, Morales H, Alzate G. Potential del hongo nativo entomopatígeno *Beauveria bassiana* como un componente de manejo integrado del picudo negro (*Cosmopolites sordidus*) en Colombia. In: XV Memorias de la Reunión de la Acobat. Medellín, Colombia, 2002, 278-283.
28. Collins PJ, Treverrow NL, Lambkin TM. Organophosphorus insecticide resistance and its management in the banana weevil borer, *Cosmopolites sordidus* (Ger.) (Coleoptera: Curculionidae) in Australia. *Crop Prot*,1991:10:215-22.