http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS

Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.154.159

Research Article
Insecticidal Effect of Weeds Extract in the Poultry Pest *Alphitobius diaperinus*

Priyantini Widiyaningrum, Ning Setiati, Sri Ngabekti, Fadilah Ngaini, Tri Dewi Yuliyanti, Yoga Hardiyanto and Suwarti

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia

Abstract

Background and Objective: *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) is the major pest in chicken farms. The control of pests using synthetic insecticides is not recommended. Besides its expensive, synthetic insecticides are proved harmful to poultry health, farmworkers and polluting the environment. To explore the sources of bioinsecticides that are cheap and eco-friendly, this study was conducted to evaluate the insecticidal effect of weeds extracts (*Chromolaena odorata*, *Ageratum conyzoides* and *Tithonia diversifolia*) against post-embryonic survival of *A. diaperinus*. **Materials and Methods:** The experimental study using a completely randomized design of 2 factors, i.e., 3 types of weeds extract and 6 levels of concentration. The insecticidal effect was observed based on the post-embryonic survival. The post-embryonic survival of larvae was evaluated for 4 weeks by measuring the number of Larvae Released (LR) from the eggs, as well as the number of Adults Emergence (AE). Data were analyzed using Variance Analysis (ANOVA) and continued with the least significant different tests ($\alpha \leq 0.5$). **Results:** The statistical analysis showed that the differences in extract concentration contributed significant influence ($\alpha \leq 0.05$) on the total number of larvae released and adult emergence. The lowest LR and AE occurred at the 40% concentration level, i.e., on the extract of *T. diversifolia*, while the highest LR and AE were found in the treatment of *C. odorata* extract. **Conclusion:** In this study, all types of extracts have an insecticidal effect on the post-embryonic survival of *A. diaperinus* but the strongest effect was found in the extract of *T. diversifolia* in 40% concentration. At last, these findings inform people that the weeds extract, especially *T. diversifolia* is possible to be developed as bioinsecticides for *A. diaperinus*.

Key words: *Alphitobius diaperinus*, insecticidal effects, post-embryonic survival, weeds extracts, adult emergence

Citation: Widiyaningrum, P., N. Setiati, S. Ngabekti, F. Ngaini and T.D. Yuliyanti, Y. Hardiyanto and Suwarti, 2022. Insecticidal effect of weeds extract in the poultry pest *Alphitobius diaperinus*. Pak. J. Biol. Sci., 25: 154-159.

Corresponding Author: Priyantini Widiyaningrum, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Most chicken farming businesses in Indonesia are small businesses managed by farmers in rural areas. The warm and humid environment in which the chicken has reared favours the development of several organisms, including the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *Alphitobius diaperinus* is one of the most common insect pests in poultry farms, where it reproduces within the littered floor, feeding on poultry manure, spilt feed and other organic material. It is the most significant pest in broiler houses worldwide[1]. This insect causes serious economic losses if not adequately controlled, because it can rapidly spread in poultry houses and it may affect birds, breeding facilities and farmworkers, Mature larvae create pupation chambers in cage wall cracks, causing structural damage. *A. diaperinus* is also difficult for poultry to digest which can cause intestinal lesions[2].

Larvae and adults are potential transmitters of several viruses, fungi and bacterial diseases (such as *Newcastle* disease, *Escherichia* spp., *Fowlpox*, *Salmonella* spp., *Aspergillus* spp. and *Campylobacter* spp.), which can lead to poultry weight loss and even death[3-7]. The presence of these insects disrupts the activity of the chicken, they often scratch the litter and feed on it, so reduction of balanced feed intake. The consumption of adult insects may injury the chicken digestive tract due to their hard elytrons. Adult *A. diaperinus* will be releasing a secretion as a defence mechanism against predators when disturbed. This secretion is a quinone compound, which is a toxic and carcinogenic substance that may cause liver lesions[8].

The use of synthetic insecticides (such as alpha-cypermethrin, spinosad and methyl-pirimiphos), is the most common method to control *A. diaperinus*, applied by spraying the floor and walls before the replacement of the litter for the next breeding cycle, to avoid direct contact with chicken[9,10]. However, the use of synthetic insecticides in addition to being expensive for small breeders is also proven to have a noticeable negative impact, often not specific and also affect non-target organisms. Negative impacts that can happen include causing pest resistance, killing non-target organisms, increasing chemical residues in the soil and agricultural products, as well as health problems for humans and livestock[11]. The loss of field efficacy of synthetic insecticides and resistance of *A. diaperinus* populations have been reported[12-14] and has led to a great interest in the development of eco-friendly insecticides. Therefore, exploration and developing a cheap and environmentally safe source of botanical insecticides (bioinsecticides) is important and indispensable.

Plants are an abundant source of secondary metabolites such as alkaloids, flavonoids, steroids, glycosides, terpenoids, tannins, saponins, quinine and phenols[15] and have the best potential as pharmaceutical drugs and biopesticides[16]. The sources of bioinsecticides that have not been widely disclosed are plants that are not of economic value but are abundantly available, such as post-harvest waste and weed crops. Weeds are a group of wild plants that are considered the main crop disruptors of agriculture, for example, *Ageratum conyzoides*, *Tithonia diversifolia* and *Chromolaena odorata* from the family Asteraceae[17]. In Indonesia, these weeds are found in corn[18] and soybeans[19]. Weeds can compete with cultivated plants in obtaining sunlight, nutrients and growing space to the detriment of the main crop. Often weeds are superior in competition because weeds have a very high growing speed, the ability to adapt to the environment and can breed vegetatively and generatively[20]. As with most plants, weeds contain secondary metabolites that serve as self-protection from the attack of predators[21]. The metabolites compound can be toxic to natural enemies, repellent, antifeedant effect or are allelopathic which can inhibit the lives of other organisms around it. The fact that weeds have the potential to be a source of bioinsecticides is expected to shift its status from a plant with no economic value to a useful plant[22].

Thus this study aims to analyze the potential of 3 weed plants (*C. odorata*, *A. conyzoides* and *T. diversifolia*) as reproductive inhibitors of poultry pest *A. diaperinus*. For small-scale farmers, bioinsecticide source plants that are easy to find around, easy to grow and environmentally friendly will be very useful information economically. The cost of obtaining and processing abundant weed crops near farmland is guaranteed to be cheaper than the cost of purchasing synthetic pesticides.

MATERIALS AND METHODS

Study area: This study was experimental design under laboratory conditions, in the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang from January-April, 2021.

Extracts preparation: The leaves of *A. conyzoides*, *T. diversifolia* and *C. odorata* were collected from Temu Kencana herbal medicinal garden, Magelang. A respective sample of weeds dried and powdered than were emulsified in with ethanol solvents 96% in ratio 1:5 for 3×24 hrs. After 72 hrs the extracted liquid was then filtered using a glass funnel given a filter paper. The filtrate was put in a vaporizing flask, then evaporated with a rotary evaporator at 45°C. The evaporation is continued by using a water bath at 50°C until all solvents were lost and produced a concentrated extract. The concentrated extract was assumed to be a 100% concentration.

Phytochemical screening: Qualitative phytochemical analysis of methanol extracts of *A. conyzoides*, *T. diversifolia* and *C. odorata* was conducted following the standard procedures. The analysis was performed through phytochemical screening of alkaloids, flavonoids, terpenoids, phenolics, steroids, saponins and tannins on extracts.

Insect preparation: Adults of *A. diaperinus* were collected from commercial poultry houses at Gunungpati Semarang Indonesia. Approximately 100 adults (males and females) were reared in the laboratory and maintained in a plastic container with a perforated cover, in the dark incubators at 27±2°C and 70±5% RH. They were fed with poultry food. Polystyrene sheet was placed in the containers, which served as a site for the larvae to pupate. The F_1 progeny were used for treatment and adults with good performance were selected for the bioassays.

Evaluation of the post embryonic survival: The survival of F_1 progeny from adult *A. diaperinus* exposed to weed extract would be observed based on the number of larvae that emergence as well as the number of larvae that successfully became adults, during 4 weeks of maintenance. A plastic cup diameter of 5 cm with 7 cm height was used as a rearing container. The experimental study using a completely randomized design of 2 factors. The 1st factor was the concentration of the extract (0, 10, 20, 30, 40, 50) and the 2nd factor was the source of the extract (*C. odorata*, *A. conyzoides*, *T. diversifolia*). Each treatment was repeated 5 times. Each experimental unit using 3 pairs of adult *A. diaperinus*. Exposure to the extract was done by dripping 150 μL per 3 pairs into the body of each insect. All of the treatments were rearing with poultry food for 4 weeks at 27±2°C and 70±5% RH. The data taken in this observation was the cumulative number of larvae released, as well as the number of larvae that emergence successfully into an adult.

Statistical analysis: The number of larvae released and the number of adult emergences was analyzed using Variance Analysis (ANOVA). If the results of the analysis showed a significant effect ($\alpha \leq 0.05$), they would be followed by a different LSD test.

RESULTS AND DISCUSSION

Phytochemical screening of weeds: The result of the phytochemical screening (Table 1) reveals that alkaloid, phenolic and tannins were positive in all ethanolic extracts of *C. odorata*, *A. conyzoides* and *T. diversifolia*. Terpenoids were detected in the ethanolic extract of *A. conyzoides* and *T. diversifolia*, while flavonoids were detected only in the ethanolic extract of *T. diversifolia* as well as steroids were detected only in the ethanolic extract of *C. odorata*. These phytochemicals may be responsible for their insecticidal properties. The presence of terpenoids revealed that the plants can act mainly as antifeedant and growth disruptor and possesses considerable toxicity toward insects. Thus, the most variation of active compounds found was in *T. diversifolia* extract.

Generally, the efficacy of bioinsecticides of plant origin that have been widely studied leads more to the efficacy of certain secondary pure metabolite isolates, for example, essential oils. However, it turns out that the bioactivity of extracts with various phytochemical compounds contained in them can be complementary or synergistic. Synergistic effects can occur when the combination of bioactive substances in the extract provides a greater effect than the effects of each component[23]. The phytochemical activity of extracts consisting of a combination of various metabolite compounds can contribute to the overall biological effects. Synergistic active compounds will increase the effectiveness of insecticides[24].

Metabolite compounds in plants that have insecticidal activity are alkaloids, flavonoids, phenols, steroids and terpenoids[25]. Alkaloids have a carbon skeleton derived from isoprenoids and those that have insect repellent activity are aconitum alkaloids and steroidal alkaloids[26]. Alkaloids, in addition to causing acute toxicity, cause disruption of biological membranes, internal damage to organs and metabolism, redox imbalances, disorders in the developmental process, reproductive disorders or causes inhibition of food intake in insects[27,28].

Terpenoids are aromatic compounds, give off a distinctive odour on each plant and can be used as insect repellents[29]. Previous studies have proven that the active compounds of terpenoid groups are toxic to insect pests. Although its mechanism of action does not directly kill the targeted insects, it contributes importantly to limiting the spread of the population. Sublethal effects include reduced fertility, shortening life, repellent effect, antifeedant, chemosterilant, growth retardant, oviposition inhibitors and attractant[30,31].

Table 1: Phytochemical screening of weeds

	Weeds		
Parameters	*C. odorata*	*A. conyzoides*	*T. diversifolia*
Alkaloids	+	+	+
Steroids	+	-	-
Terpenoids	-	+	+
Flavonoids	-	-	++
Phenolic	+	+	+
Saponins	-	-	-
Tannins	+	+	+

+: Detected, ++: More detected and -: Undetectable

Table 2: Total number of larvae released and adult emergence

Extract concentration (%)	*C. odorata*	*A. conyzoides*	*T. diversifolia*	Average
Total number of larvae released				
0	69±22.057	69±22.057	69±22.057	69±22.057[a]
10	69±14.533	63±7.301	54±16.263	62±12.699[ab]
20	53±11.000	55±18.823	49±14.832	52±14.855[bc]
30	50±20.401	56±13.255	40±14.536	49±16.064[cd]
40	53±19.766	43±8.933	22±6.671	39±11.790[de]
50	47±9.555	36±13.183	19±4.764	34±9.167[e]
Average	57±16.218[a]	54±13.925[a]	42±13.187[b]	
Total number of adult emergence				
0	67±21.078	67±21.078	67±21.078	67±21.078[a]
10	66±14.433	59±9.524	48±15.754	58±13.237[b]
20	51±10.780	51±20.732	45±12.798	49±14.770[c]
30	47±17.649	53±12.689	36±14.536	45±14.958[c]
40	49±18.398	37±9.680	17±6.066	34±11.381[d]
50	42±8.136	29±11.323	12±2.550	28±7.336[d]
Average	54±15.079[a]	49±14.171[a]	37±12.130[b]	

Different letter in the same printed column shows a significant difference at 5% significance level and based on LSD *post-hoc* test

Therefore, it is necessary to examine the effect of the phytochemical compounds of 3 types of weeds on the survival of *A. diaperinus* larvae.

Evaluation of the post embryonic survival: The postembryonic survival of *A. diaperinus* was measured based on the number of larvae released as well as the number of larvae that successfully metamorphosed into adults (Table 2).

The statistical analysis showed that the differences in extract concentration contributed significant influence ($\alpha \leq 0.05$) on the total number of Larvae Released (LR) and Adult Emergence (AE). The lowest LR and AE occurred at the 40% concentration level, i.e., on the extract of *T. diversifolia*, while the highest LR and AE were found in the treatment of *C. odorata* extract. Within 4 weeks, the average number of larvae and the number of adults found in the treatment of *A. conyzoides* extracts didn't significant from the treatment of *C. odorata* ($\alpha \geq 0.05$) but both significant to the number of larvae and adults in the *T. diversifolia* treatment. The higher the concentration of the extract is, the less the number of larvae will be released. Apparently, the higher the concentration of extracts that are in direct contact with the body of insects is, the greater the chance of the extract being absorbed into the body and disrupting the reproductive tract. Yasmin *et al.*[32] state that the direct contact, the active compound of the extract can penetrate the insect body through its spiracle organs. The entry of the active compound of neem leaves into the body of *A. diaperinus* disrupts the oviposition tract and inhibits the process of laying eggs (oviposition deterrent).

Ingrid *et al.*[1] also found that the effects of *A. conyzoides* extract have affected the histopathology of the reproductive tract of locusts *Zonocerus variegatus.* On the contrary, Green *et al.*[33] found something different because in their research *T. diversifolia* extract did not affect the egg-laying ability of *C. maculatus* ($p>0.05$). Belmain *et al.*[34] argue that the efficacy of each extract may vary greatly, influenced by plant genetics and growing environmental differences. The dominant components contained in the extract, the species of test insects, the concentration as well as the way the active compounds of the extract entering the body will also affect its efficacy. In this study, a decrease in the number of larvae and the number of adult ticks shows that the three weed extracts of *C. odorata*, *A. conyzoides* and *T. diversifolia* can be used to suppress *A. diaperinus* population. *Tithonia diversifolia* extract provides the best results compared to the other 2 weed extracts.

CONCLUSION

All types of extracts have an insecticidal effect on the post-embryonic survival of *A. diaperinus* but the strongest effect was found in the extract of *T. diversifolia* in 40% concentration. At last, these findings inform people that the weeds extract, especially *T. diversifolia* is possible to be developed as bioinsecticides for *A. diaperinus*.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of bioactive substances in the weeds extract and provides a greater insecticidal effect than the effects of each component. At least, *T. diversifolia* expressed the strongest effect because it contains 5 types of secondary metabolites, more than the other 2 weeds. This study will help the researcher to uncover the botanical insecticides that are cheap and eco-friendly that many researchers were not able to explore.

ACKNOWLEDGMENT

The author would like to thank Universitas Negeri Semarang for funding this research, through the 2021 UNNES DIPA fund with Number: 242.26.4/UN37/PPK.3.1/2021.

REFERENCES

1. Ingrid, D.T., S.R. Akwanjoh and M. Yacouba, 2020. Histopathological effects of *Ageratum conyzoides* (Asteraceae) on the male reproductive system of the pest grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae). J. Entomol. Zool. Stud., 8: 643-647.
2. Rumbos, C.I., I.T. Karapanagiotidis, E. Mente and C.G. Athanassiou, 2019. The lesser mealworm *Alphitobius diaperinus*: A noxious pest or a promising nutrient source? Rev. Aquacult., 11: 1418-1437.
3. Crippen, T.L., C.L. Sheffield, R.C. Beier and D.J. Nisbet, 2018. The horizontal transfer of *Salmonella* between the lesser mealworm (*Alphitobius diaperinus*) and poultry manure. Zoonoses Public Health, 65: e23-e33.
4. da Silva Soares, C.E., A. Weber and V.M. Scussel, 2018. Stereo and scanning electron microscopy characteristics of poultry breeding beetle (*Alphitobius diaperinus*)-a filamentous toxigenic fungi carrier. Emir. J. Food Agric., 30: 150-156.
5. Del Valle, E.E., L.S. Frizzo, M. Malmierca, M.V. Zbrun, P. Lax and M.E. Doucet, 2016. Biological control of *Alphitobius diaperinus* with *Steinernema rarum* CUL and *Heterorhabditis bacteriophora* SMC and feasibility of application in rice hull. J. Pest Sci., 89: 161-170.
6. Arunraj, C., S.K. Thomas and P.M. Nirdev, 2013. Lesser mealworm, *Alphitobius diaperinus* (panzer, 1797) (Coleoptera: Tenebrionidae) menace in poultry farms in south India. J. Biopesticides, 6: 84-86.
7. Chernaki-Leffer, A.M., D.R. Sosa-Gómez, L.M. Almeida and I.D.O.N. Lopes, 2011. Susceptibility of *Alphitobius diaperinus* (Panzer) (Coleoptera, Tenebrionidae) to cypermethrin, dichlorvos and triflumuron in southern Brazil Rev. Bras. Entomol., 55: 125-128.
8. Stejskal, V., J. Hubert and Z. Li, 2018. Human health problems and accidents associated with occurrence and control of storage arthropods and rodents. In: Recent Advances in Stored Product Protection, Athanassiou, C. and F. Arthur (Eds.), Springer Berlin Heidelberg, New York, ISBN-13: 978-3-662-56125-6, pp: 19-43.
9. Wolf, J., M. Potrich, E.R. Lozano, A. Gouvea and C.S. Pegorini, 2015. Combined physical and chemical methods to control lesser mealworm beetles under laboratory conditions. Poult. Sci., 94: 1145-1149.
10. Zafeiriadis, S., M.K. Sakka and C.G. Athanassiou, 2021. Efficacy of contact insecticides for the control of the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). J. Stored Prod. Res., Vol. 92. 10.1016/j.jspr.2021.101817.
11. Gill, H.K. and H. Garg, 2014. Pesticides: Environmental impacts and management strategies. In: Pesticides: Toxic Aspects, Soloneski, S. (Ed.), IntechOpen Limited, London, ISBN-13: 978-953-51-1217-4, pp: 187-230.
12. Lambkin, T.A. and M.J. Furlong, 2011. Metabolic mechanisms only partially explain resistance to pyrethroids in Australian broiler house populations of lesser mealworm (Coleoptera: Tenebrionidae). J. Econ. Entomol., 104: 629-635.
13. Arena, J.S., A.B. Omarini, M.P. Zunino, M.L. Peschiutta, M.T. Defagó and J.A. Zygadlo, 2018. Essential oils from *Dysphania ambrosioides* and *Tagetes minuta* enhance the toxicity of a conventional insecticide against *Alphitobius diaperinus*. Ind. Crops Prod., 122: 190-194.
14. Hickmann, F., A.F. de Morais, E.S. Bronzatto, T. Giacomelli, J.V.C. Guedes and O. Bernardi, 2018. Susceptibility of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), from broiler farms of southern Brazil to insecticides. J. Econ. Entomol., 111: 980-985.
15. Jung, H.W., T.J. Tschaplinski, L. Wang, J. Glazebrook and J.T. Greenberg, 2009. Priming in systemic plant immunity. Science, 324: 89-91.
16. Rioba, N.B. and P.C. Stevenson, 2017. *Ageratum conyzoides* L. for the management of pests and diseases by small holder farmers. Ind. Crops Prod., 110: 22-29.
17. Folorunso, A.E. and O.D. Awosode, 2013. Comparative anatomy of invasive and non-invasive species in the family Asteraceae in Nigeria. Int. J. Bio. Chem. Sci., 7: 1804-1819.
18. Kurniadie, D., U. Umiyati and D. Widayat, 2016. Weed survey in sweet corn (*Zea mays saccharata* Sturt.) in regency of sumedang and bandung Indonesia. Asian J. Crop Sci., 8: 66-70.

19. Hasanuddin, S. Hafsah, E. Nurahmi, E. Hayati, S.W. Migawati, J. Bobihoe and D.S. Aryani, 2021. The application of different mulches and its effect on soybean yield. IOP Conf. Ser.: Earth Environ. Sci. Vol. 644. 10.1088/1755-1315/644/1/012069.
20. Prabha, S., A. Yadav, A. Kumar, A. Yadav and H.K. Yadav *et al*., 2016. Biopesticides an alternative and eco-friendly source for the control of pests in agricultural crops. Plant Arch., 16: 902-906.
21. Mkenda, P., R. Mwanauta, P.C. Stevenson, P. Ndakidemi, K. Mtei and S.R. Belmain, 2015. Extracts from field margin weeds provide economically viable and environmentally benign pest control compared to synthetic pesticides. PLoS One, Vol. 10. 10.1371/journal.pone.0143530.
22. Nenaah, G.E., 2014. Bioactivity of powders and essential oils of three Asteraceae plants as post-harvest grain protectants against three major coleopteran pests. J. Asia-Pac. Entomol., 17: 701-709.
23. Richards, L.A., A.E. Glassmire, K.M. Ochsenrider, A.M. Smilanich, C.D. Dodson, C.S. Jeffrey and L.A. Dyer, 2016. Phytochemical diversity and synergistic effects on herbivores. Phytochem. Rev., 15: 1153-1166.
24. Ventrella, E., P. Marciniak, Z. Adamski, G. Rosiński and S. Chowański *et al*., 2015. Cardioactive properties of solanaceae plant extracts and pure glycoalkaloids on *Zophobas atratus*. Insect Sci., 22: 251-262.
25. Oriyomi, O.V., 2018. Phytochemical biopesticides. In: Phytochemistry, Egbuna, C., J.C. Ifemeje, S. Kumar and N. Sharif (Eds.), (Edn. 1st), Apple Academic Press, New York, ISBN-13: 9780429426155, pp: 303-324.
26. Saxena, M., J. Saxena, R. Nema, D. Singh and A. Gupta, 2013. Phytochemistry of medicinal plants. J. Pharmacogn. Phytochem., 1: 168-182.
27. Chowański, S., Z. Adamski, P. Marciniak, G. Rosiński and E. Büyükgüzel *et al*., 2016. A review of bioinsecticidal activity of solanaceae alkaloids. Toxins, Vol. 60. 10.3390/toxins8030060.
28. Singh, S.K., 2018. Explorations of plant's chemodiversity: Role of nitrogen-containing secondary metabolites in plant defense. In: Molecular Aspects of Plant-Pathogen Interaction. Singh, A. and I. Singh (Eds.), (Edn. 1st), Springer, Cham, Singapore, ISBN-13: 978-981-10-7370-0, pp: 309-332.
29. Spochacz, M., S. Chowański, K. Walkowiak-Nowicka, M. Szymczak and Z. Adamski, 2018. Plant-derived substances used against beetles-pests of stored crops and food-and their mode of action: A review. Compr. Rev. Food Sci. Food Saf., 17: 1339-1366.
30. Kortbeek, R.W.J., M. van der Gragt and P.M. Bleeker, 2019. Endogenous plant metabolites against insects. Eur. J. Plant Pathol., 154: 67-90.
31. Hikal, W.M., R.S. Baeshen and H.A.H. Said-Al Ahl, 2017. Botanical insecticide as simple extractives for pest control. Cogent Biol., Vol. 3. 10.1080/23312025.2017.1404274.
32. Yasmin, A., N.C. Ghorai and A. Islam, 2016. Effects of phytochemicals in the postembryonic development of the lesser mealworm-*Alphitobius diaperinus* (panzer) (Insecta: Coleoptera: Tenebrionidae). Int. J. Recent Res. Life Sci., 3: 25-33.
33. Green, P.W.C., S.R. Belmain, P.A. Ndakidemi, I.W. Farrell and P.C. Stevenson, 2017. Insecticidal activity of *Tithonia diversifolia* and *Vernonia amygdalina*. Ind. Crops Prod., 110: 15-21.
34. Belmain, S.R., B.A. Amoah, S.P. Nyirenda, J.F. Kamanula and P.C. Stevenson, 2012. Highly variable insect control efficacy of *Tephrosia vogelii* chemotypes. J. Agric. Food Chem., 60: 10055-10063.