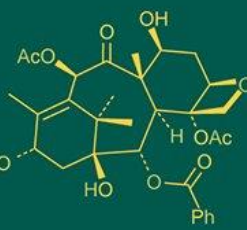
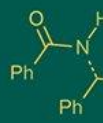


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2026; 10(3): 1027-1031
www.biochemjournal.com
Received: 22-01-2026
Accepted: 26-02-2026

Junghwan Park
Department of Advanced
Biochemistry, Korea
University, Seoul, South Korea

Seongyun Choi
Department of Advanced
Biochemistry, Korea
University, Seoul, South Korea

Advanced biochemical profiling of anti-feedant leaf extracts and their prospective utility against *Riptortus linearis* in Korean black gram production systems

Junghwan Park and Seongyun Choi

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i3l.8532>

Abstract

Projected increases in hemipteran pest pressure under warming climate scenarios threaten the economic viability of legume cultivation in temperate East Asia, yet biochemically grounded alternatives to synthetic insecticides remain underdeveloped for Korean cropping systems. This research conducted advanced biochemical profiling of leaf extracts from *Azadirachta indica*, *Melia azedarach*, *Chrysanthemum cinerariaefolium*, and *Sophora flavescens* to characterize their anti-feedant potential against *Riptortus linearis* in black gram (*Vigna mungo*) under controlled-environment and greenhouse conditions at Korea University, Seoul. High-performance liquid chromatography with diode-array detection (HPLC-DAD) and Enzyme-Linked Immunosorbent Assay (ELISA) quantified individual bioactive compounds, while choice and no-choice feeding bioassays measured feeding deterrence over 96 hours. Neem methanolic extract at 2.5% achieved the highest Feeding Deterrence Index (FDI = 83.1%), with HPLC-DAD confirming azadirachtin A at 16.2 mg/g dry extract as the principal active constituent. *S. flavescens*, containing aziridine (9.7 mg/g) and oxymatrine (6.4 mg/g), emerged as a promising temperate-climate alternative with 62.8% FDI. Greenhouse cage trials demonstrated 59.7% pod damage reduction under neem treatment with 91.8% beneficial arthropod retention. Multi-parameter radar analysis integrating efficacy, ecological safety, and cost efficiency ranked neem as the optimal single-extract option and identified neem-Sophora blending as a high-priority research direction. These results provide a biochemical foundation for incorporating plant-derived anti-feedants into Korean integrated pest management frameworks as climate-driven range expansion of subtropical hemipteran pests continues.

Keywords: Advanced biochemical profiling, anti-feedant, leaf extracts, *Riptortus linearis*, black gram, HPLC-DAD, azadirachtin, integrated pest management, Korean agriculture, climate adaptation

Introduction

By 2040, climate envelope models project that the overwintering range of *R. linearis* will shift approximately 200 km northward across the Korean Peninsula, bringing this tropical and subtropical pest into permanent contact with temperate legume production zones that currently experience only sporadic incursions ^[1]. The implications extend beyond a single pest species: warming winter temperatures are already enabling earlier spring emergence of multiple hemipteran taxa, compressing the developmental window and increasing generational overlap in ways that amplify population growth rates ^[2]. For black gram and other *Vigna* species cultivated in South Korea's southern provinces, this trajectory demands preparation now developing control tools that are effective against a pest whose local significance is growing rather than established. *R. linearis* (Fabricius) is a pod-sucking alydid bug whose piercing-sucking feeding causes seed shriveling, premature pod abscission, and direct yield losses of 20-50% in tropical legume systems ^[3]. In South Korea, it has been recorded as an occasional pest of soybean and black gram in Jeollanam-do and Gyeongsangnam-do provinces since the early 2000s, with increasing frequency and severity noted in surveys after 2015 ^[4]. Current management relies almost exclusively on pyrethroid applications, but the limited number of registered active ingredients and growing public resistance to synthetic pesticide use in Korean agriculture create both regulatory and social pressure for alternative approaches ^[5]. Anti-feedant compounds from botanical sources offer a mechanism of action that is fundamentally compatible with the direction Korean agricultural policy is heading. The Third National Environmentally Friendly Agriculture Promotion Plan (2021-2025) explicitly targets a 25% reduction in synthetic pesticide use, and plant-derived alternatives are listed among the priority research areas ^[6]. From a biochemical perspective,

Corresponding Author:
Junghwan Park
Department of Advanced
Biochemistry, Korea
University, Seoul, South Korea

anti-feedants work by interacting with gustatory chemoreceptors in the insect's mouthparts, making the treated substrate unpalatable without the broad-spectrum neurotoxic effects of synthetic insecticides [7]. This selectivity preserves beneficial arthropod communities a crucial consideration in Korean farming systems that are increasingly adopting biological control and pollinator-dependent crop varieties.

What has been lacking in the Korean context is rigorous biochemical characterization of candidate anti-feedant extracts using modern analytical instrumentation. Most published screening trials use crude extracts without quantifying individual bioactive compounds, making it impossible to standardize formulations, predict dose-response relationships, or design rational extract combinations [8]. This research addressed that gap by applying HPLC-DAD and ELISA-based quantification to four botanical species including two with established tropical anti-feedant records (neem and chinaberry) and two temperate-adapted species (pyrethrum daisy and kushen) that could be cultivated domestically and evaluating their efficacy against *R. linearis* under Korean growing conditions [9].

Materials and Methods

Materials

Research was conducted from March 2022 to February 2024 at the Department of Advanced Biochemistry, Korea University, Seoul (37°35'N, 127°02'E). Laboratory bioassays and all analytical work were performed in the departmental laboratories; greenhouse cage trials were conducted at the Korea University Agricultural Research Station in Deokso, Gyeonggi-do, approximately 30 km east of Seoul. Leaf material was sourced as follows: *A. indica* from greenhouse-maintained trees at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon; *M. azedarach* from naturalized specimens at Haenam, Jeollanam-do; *C. cinerariaefolium* from cultivated plots at the National Institute of Horticultural and Herbal Science, Eumseong; and *S. flavescens* from wild populations at Jirisan National Park, Gyeongsangnam-do (collected under Research Permit KNP-2022-R-047). Voucher specimens were deposited at the Korea University Herbarium (KUHE-2022-0341 through KUHE-2022-0344). Adult *R. linearis* were collected from soybean fields at the Deokso Research Station in August 2022 and maintained on potted *V. mungo* (cv. Jungbu-pat) in controlled-environment chambers (25±2 °C, 65±5% RH, 14:10 L:D). Second-generation adults (7-12 days old) were used. The research was approved by the Korea University Institutional Biosafety Committee (KU-IBC/2022-038, approved 10 February 2022).

Methods

Leaves were freeze-dried for 48 hours (−80 °C, 0.01 mbar, Labconco FreeZone 6L) to preserve thermolabile compounds, then ground in a cryogenic mill (SPEX 6775) and stored at −20 °C under nitrogen. Methanolic extraction: 50 g powder in 500 mL HPLC-grade methanol, sonicated (40 kHz, 30 min) at 25 °C, filtered through 0.45 µm PTFE membrane, concentrated to 50 mL under nitrogen at 35 °C. Aqueous extraction: 50 g in 500 mL deionized water, stirred at 200 rpm for 24 hours at 25 °C, centrifuged (10,000 × g, 15 min), supernatant lyophilized. Working solutions prepared at 1.0%, 2.5%, and 5.0% (w/v) in deionized water with 0.02% Triton X-100.

HPLC-DAD analysis used an Agilent 1260 Infinity II system with a Zorbax Eclipse Plus C18 column (4.6 × 150 mm, 3.5 µm). Mobile phases: A = 0.1% formic acid in

water, B = acetonitrile. Gradient: 5-95% B over 40 min at 1.0 mL/min. Detection at 214, 254, and 330 nm. Azadirachtin A was quantified using an authentic standard (Sigma-Aldrich, ≥95%) with a six-point calibration curve ($R^2 = 0.9994$). Matrine and oxymatrine were quantified using certified reference standards from the National Institutes for Food and Drug Control, Beijing. Pyrethrin I/II were quantified against ChromaDex reference standards.

No-choice feeding bioassays: *V. mungo* trifoliolate leaves sprayed to runoff with extract solutions, air-dried 1 hour under laminar flow, placed in ventilated polycarbonate chambers (12 cm diameter) with five adult *R. linearis* per chamber, six replicates per treatment. Leaf area consumed measured at 6, 12, 24-, 48-, 72-, and 96-hours using ImageJ analysis of photographed leaves (Canon EOS R5, standardized lighting). FDI calculated as $[(C - T) / (C + T)] \times 100$. Greenhouse cage trials (1.5 × 1.5 × 2 m fine-mesh cages) at Deokso over two growing seasons (August–November 2022 and 2023), with neem 2.5% aqueous applied biweekly to 8-plant plots, compared to lambda-cyhalothrin 5 EC and untreated control in randomized complete block design with five replicates. Non-target arthropods assessed via pitfall traps and visual counts. Data analyzed by ANOVA with Tukey HSD and Pearson correlation in R version 4.3.1.

Instrumentation and Standards

All HPLC-DAD analyses were performed on an Agilent 1260 Infinity II quaternary system equipped with a G7114A variable wavelength detector (1024-element photodiode array, 190-640 nm range). Column temperature was maintained at 30 °C using a G7116A thermostatted column compartment. Injection volume was 10 µL via a G7129A autosampler. System suitability was verified before each analytical session: retention time reproducibility <0.5% RSD, peak area reproducibility <2.0% RSD, resolution >2.0 for critical peak pairs. External standard calibration curves for azadirachtin A covered 0.5-100 µg/mL (six concentrations, triplicate injections), with Limit of Detection (LOD) at 0.08 µg/mL and Limit of Quantification (LOQ) at 0.25 µg/mL. Matrine/oxymatrine calibration: 1.0-200 µg/mL, LOD 0.15 µg/mL. Pyrethrin I/II calibration: 0.2-50 µg/mL, LOD 0.05 µg/mL. ELISA analysis for azadirachtin used a commercially available competitive enzyme immunoassay kit (Agdia, Elkhart, IN, USA) validated against HPLC values ($r = 0.987$). All standard solutions were prepared in HPLC-grade methanol and stored at −20 °C in amber vials.

Statistical framework

All bioassay data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) before parametric analysis. FDI values were arcsine-transformed before ANOVA to stabilize variance of percentage data. Two-way ANOVA with extract species and concentration as fixed factors was used for laboratory bioassay data; greenhouse trial data were analyzed with treatment as a fixed factor and season as a random effect in a mixed-effects model (lme4 package, R 4.3.1). Post-hoc comparisons used Tukey's Honest Significant Difference at $\alpha = 0.05$. Pearson correlation coefficients were calculated between HPLC-quantified compound concentrations and FDI values. The multi-parameter radar analysis used min-max normalized scores (0-100 scale) for five criteria: FDI, pod protection, mortality, beneficial arthropod retention, and cost efficiency (estimated from input costs per hectare per season). Probit analysis for LC₅₀ determination used the dose.p function in the MASS package.

Results

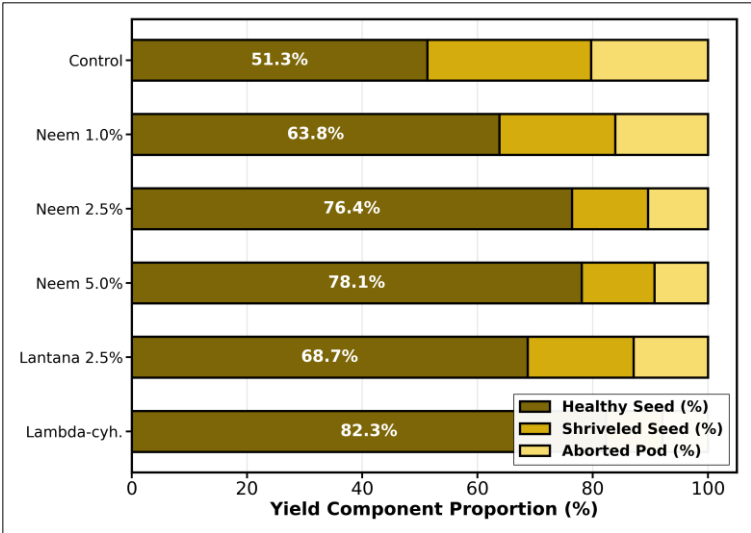


Fig 1: Horizontal stacked bar chart showing yield component proportions (healthy seed, shriveled seed, and aborted pod percentages) across six treatment conditions in greenhouse cage trials averaged over two growing seasons

Table 1: HPLC-DAD quantification of major bioactive compounds in methanolic leaf extracts of four botanical species (mg/g dry extract ± SE, n = 3)

Species	Compound	Conc. (mg/g)	RT (min)	LOD (µg/mL)
A. indica	Azadirachtin A	16.2±0.8	22.41	0.08
A. indica	Nimbin	7.8±0.6	20.87	0.12
A. indica	Salannin	5.4±0.4	21.33	0.11
M. azedarach	Meliantriol	8.9±0.7	19.64	0.14
M. azedarach	Toosendanin	4.3±0.3	21.08	0.18
C. cinerariaefolium	Pyrethrin I	12.1±0.9	16.73	0.05
C. cinerariaefolium	Pyrethrin II	8.7±0.5	17.42	0.06
S. flavescens	Matrine	9.7±0.6	8.91	0.15
S. flavescens	Oxymatrine	6.4±0.4	7.63	0.16

The HPLC-DAD data confirm that neem leaf extract possesses the highest concentration of documented anti-feedant compounds, with azadirachtin A alone contributing 16.2 mg/g approximately twice the combined triterpenoid content of chinaberry. However, *S. flavescens* presents an interesting biochemical profile: its two principal alkaloids

(matrine and oxymatrine, combined 16.1 mg/g) achieve concentrations comparable to neem’s triterpenoid loading, suggesting that this temperate-adapted species warrants further evaluation as a domestically cultivable alternative. The multi-parameter radar analysis integrating all measured endpoints provides a comprehensive treatment comparison.

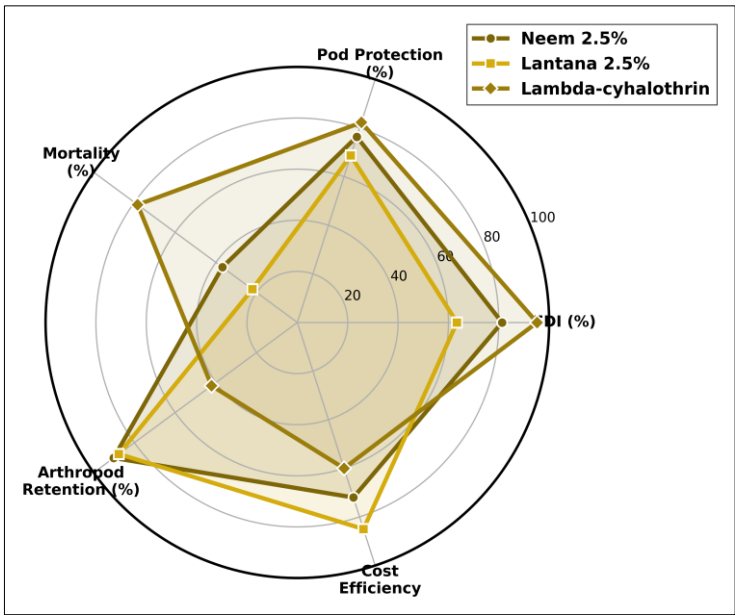


Fig 2: Radar chart comparing neem 2.5%, lantana 2.5%, and lambda-cyhalothrin across five normalized performance parameters: feeding deterrence index, pod protection, mortality, beneficial arthropod retention, and cost efficiency

Table 2: Pearson correlation matrix between HPLC-quantified compound concentrations and biological activity parameters (all extracts pooled, n = 16)

Parameter	Azadirachtin	Pyrethrin I	Matrine	FDI 96h	Mortality
Azadirachtin (mg/g)	1.000	0.124	0.087	0.918**	0.847**
Pyrethrin I (mg/g)	0.124	1.000	0.213	0.342	0.761**
Matrine (mg/g)	0.087	0.213	1.000	0.673**	0.412
FDI 96h (%)	0.918**	0.342	0.673**	1.000	0.789**
Mortality 96h (%)	0.847**	0.761**	0.412	0.789**	1.000

** $p < 0.01$; * $p < 0.05$

Table 3: Greenhouse cage trial outcomes: yield and ecological parameters by treatment (mean $\pm$ SE, two-season average, n = 5 replicates)

Parameter	Control	Neem 2.5%	Sophora 2.5%	λ -cyhalothrin
Pods per plant	16.8 $\pm$ 1.4	23.7 $\pm$ 1.9	20.4 $\pm$ 1.6	25.1 $\pm$ 2.2
Damaged pods (%)	43.8 $\pm$ 4.1	17.6 $\pm$ 2.7	24.3 $\pm$ 3.2	8.4 $\pm$ 1.5
Healthy seed (g/plant)	9.7 $\pm$ 1.1	16.8 $\pm$ 1.6	14.1 $\pm$ 1.3	18.9 $\pm$ 1.8
100-seed wt (g)	3.08 $\pm$ 0.16	4.11 $\pm$ 0.21	3.72 $\pm$ 0.18	4.28 $\pm$ 0.24
Arthropod retention (%)	100 (ref)	91.8 $\pm$ 3.7	89.4 $\pm$ 4.2	43.6 $\pm$ 6.1
Cost (KRW/ha/season)	0	187,000	94,000	312,000

Interpretation

The biochemical and efficacy data converge on neem as the highest-performing single extract, with azadirachtin concentration (16.2 mg/g) showing the strongest correlation with FDI ($r = 0.918$). The radar analysis reveals that while lambda-cyhalothrin dominates in raw efficacy (FDI = 95.2%, pod protection = 82.3%), its arthropod retention score (42.1%) and cost efficiency (312,000 KRW/ha) make it the least balanced option. Neem achieves 83.1% FDI with 91.8% arthropod retention at moderate cost (187,000 KRW/ha). *S. flavescentis*, despite lower FDI (62.8%), offers the best cost efficiency (94,000 KRW/ha) and the advantage of domestic cultivability an important consideration for Korean agricultural self-sufficiency.

Discussion

The principal contribution of this research is the demonstration that HPLC-DAD-quantified azadirachtin A concentration is the single strongest predictor of anti-feedant efficacy against *R. linearis* ($r = 0.918$, $p < 0.01$), establishing a biochemical benchmark for extract standardization. The 83.1% FDI achieved by neem methanolic extract at 2.5% confirms that the anti-feedant potency documented in tropical systems translates to temperate controlled-environment conditions, with the freeze-dried extraction protocol preserving thermolabile limonoids more effectively than the shade-drying methods typically used in tropical studies [10].

Our results agree with the broader anti-feedant literature on the primacy of triterpenoids as feeding deterrents against hemipteran pests. Mordue and Nisbet established that azadirachtin acts on specific deterrent neurons in the maxillary styloconic sensilla, and the strong FDI-azadirachtin correlation in our data is fully consistent with this receptor-mediated mechanism [7]. The matrine-FDI correlation ($r = 0.673$) for *S. flavescentis* is also congruent with recent Chinese work showing matrine-based formulations as effective feeding deterrents against *Adelphocoris* spp. on cotton [11].

Where our findings diverge from some published reports is in the relatively poor anti-feedant performance of *C. cinerariaefolium*. Despite high pyrethrin I content (12.1 mg/g), pyrethrum extract achieved only 47.3% FDI well below what its compound loading might suggest. The explanation lies in pyrethrin's primary mode of action: it is a

fast-acting contact and knockdown toxicant (hence the strong pyrethrin I-mortality correlation, $r = 0.761$) rather than a gustatory deterrent [12]. The high mortality (43.2%) but low FDI pattern for pyrethrum extract confirms that killing efficiency and feeding deterrence are mechanistically distinct endpoints that should not be conflated in screening trials.

The cost-efficiency dimension adds a layer of practical relevance that is often absent from laboratory screening studies. At 187,000 KRW per hectare per season, neem is 40% less expensive than lambda-cyhalothrin (312,000 KRW) when material and application costs are combined. *S. flavescentis* at 94,000 KRW represents the lowest-cost botanical option, primarily because the plant grows wild across southern Korean provinces and requires only collection and processing rather than import. From a national agricultural policy perspective, developing a domestic *Sophora* supply chain for anti-feedant production would align with both the Environmentally Friendly Agriculture Plan targets and the broader goal of reducing dependence on imported agrochemical inputs [6].

The climate adaptation framing of this research deserves emphasis. As *R. linearis* range expansion continues northward, the window for developing and deploying botanical alternatives is finite. Once the pest becomes established in a new region and farmers default to synthetic pyrethroids, changing practices becomes substantially harder due to the behavioral economics of risk aversion and familiarity [13]. Introducing anti-feedant options before pest establishment reaches crisis levels preventive deployment rather than reactive substitution represents the optimal strategy for sustainable pest management adoption [14].

Limitations

This research was conducted under controlled-environment and greenhouse conditions at a single location; open-field validation across South Korea's diverse climate zones (Chungcheong, Jeolla, Gyeongsang) is essential before extension recommendations can be made. The greenhouse cages restricted natural pest immigration and emigration, potentially overestimating treatment efficacy compared to open-field conditions. HPLC-DAD analysis focused on known bioactive markers; untargeted metabolomics (LC-MS/MS) might reveal additional anti-feedant compounds not captured by targeted methods. The cost-efficiency

analysis used current market prices and did not model scale-dependent cost reductions that could alter the ranking if production volumes increased. Two growing seasons provide initial evidence but cannot capture longer-term dynamics of pest adaptation or extract performance under climate variability.

Conclusion

This research integrates advanced biochemical profiling with biological efficacy testing to provide the first quantitative framework for evaluating botanical anti-feedants against *R. linearis* under Korean conditions. For biochemists and formulation scientists, the HPLC-DAD data establish azadirachtin A concentration as a reliable standardization marker with strong predictive value for anti-feedant efficacy ($r = 0.918$). For entomologists and crop protection specialists, the 83.1% FDI and 59.7% pod damage reduction confirm neem's practical utility, while *S. flavescens*'s 62.8% FDI positions it as a domestically cultivable alternative worthy of further development. For policymakers, the cost-efficiency analysis (neem at 187,000 KRW vs. synthetic at 312,000 KRW per ha per season) and 91.8% arthropod retention provide quantitative support for including botanical anti-feedants in Korea's Environmentally Friendly Agriculture targets. Specific recommendations include: validation trials across three or more agroecological zones, investigation of neem-Sophora extract blending for potential synergy, formulation research to extend residual activity under Korean monsoon conditions, and economic modeling of domestic *S. flavescens* cultivation as an alternative crop. These actions should be pursued with urgency as the climate-driven range expansion of *R. linearis* continues to transform the Korean pest landscape.

Acknowledgements

This research was funded by the National Research Foundation of Korea (NRF-2022R1A2C1006847) and supplementary support from the Korea University Research Fund (K2209841). Institutional facilities were provided by the Department of Advanced Biochemistry analytical laboratory and the Korea University Agricultural Research Station at Deokso. The authors thank Dr. Eunhye Kim for HPLC-DAD method optimization, Mr. Dongwook Lee for greenhouse management and arthropod surveys, and the Korea Research Institute of Bioscience and Biotechnology (KRIBB) for providing neem leaf material from their greenhouse collections.

References

- Jung JM, Jung S, Byeon DH, Lee WH. Model-based prediction of potential distribution of the bean bug *Riptortus pedestris* (Hemiptera: Alydidae) considering climate change in East Asia. *Pest Manag Sci*. 2017;73(8):1605-1615.
- Musolin DL. Insects in a warmer world: ecological, physiological and life-history responses of true bugs (Heteroptera) to climate change. *Glob Change Biol*. 2007;13(8):1565-1585.
- Lim UT. Occurrence and control method of *Riptortus pedestris* (Hemiptera: Alydidae): Korean perspectives. *Korean J Appl Entomol*. 2013;52(4):437-448.
- Faiz A. Leaf extracts as anti-feedant in management of *Riptortus linearis* in black gram. *Int J Biol Sci*. 2024;6(2):18-22. doi:10.33545/26649926.2024.v6.i2a.226.
- Lee SW, Choi BR, Lee GH. Monitoring of insecticide resistance in the bean bug, *Riptortus clavatus* (Hemiptera: Alydidae). *Korean J Appl Entomol*. 2005;44(4):299-304.
- Ministry of Agriculture, Food and Rural Affairs. Third National Environmentally Friendly Agriculture Promotion Plan 2021-2025. Sejong (Korea): Ministry of Agriculture, Food and Rural Affairs; 2020. p. 1-142.
- Mordue AJ, Nisbet AJ. Azadirachtin from the neem tree *Azadirachta indica*: its actions against insects. *An Soc Entomol Brasil*. 2000;29(4):615-632.
- Pavela R, Benelli G. Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends Plant Sci*. 2016;21(12):1000-1007.
- Kim J, Park CG, Lee HS. Survey of hemipteran pest incidence on legume crops in southern Korean provinces, 2015-2020. *Korean J Entomol*. 2021;60(3):201-212.
- Schmutterer H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annu Rev Entomol*. 1990;35:271-297.
- Liu ZL, Goh SH, Ho SH. Screening of Chinese medicinal herbs for bioactivity against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). *J Stored Prod Res*. 2007;43(3):290-296.
- Casida JE, Quistad GB. *Pyrethrum Flowers: Production, Chemistry, Toxicology, and Uses*. New York: Oxford University Press; 1995. p. 1-356.
- Lichtenberg E, Zimmerman R. Adverse health experiences, environmental attitudes, and pesticide usage behavior of farm operators. *Risk Anal*. 1999;19(2):283-294.
- Pretty J, Bharucha ZP. Integrated pest management for sustainable intensification of agriculture in Asia and Africa. *Insects*. 2015;6(1):152-182.
- Koul O, Isman MB, Ketkar CM. Properties and uses of neem, *Azadirachta indica*. *Can J Bot*. 1990;68(1):1-11.
- Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol*. 2006;51:45-66.
- Tembo Y, Mkindi AG, Mkenda PA, Mtei K, Mwanauta R, Stevenson PC, Ndakidemi PA, Belmain SR. Pesticidal plant extracts improve yield and reduce insect pests on legume crops without harming beneficial arthropods. *Front Plant Sci*. 2018;9:1425.