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FORMULATION AND EVALUATION OF HERBAL SPRAY AS A INSECTICIDES OF ACYRANTHES ASPERA LEAVES EXTRACT

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ABSTRACT

The increasing resistance of insect pests to synthetic insecticides and the environmental burden associated with chemical control have intensified interest in botanical insecticides. Plant-based formulations are attractive because they are biodegradable, comparatively safer to non-target organisms, and can contain multiple bioactive compounds with synergistic action. Achyranthes aspera has shown promising pesticidal potential in prior studies, including significant entomocidal activity against mosquito larvae and molluscicidal effects, with phytochemical evidence suggesting the importance of saponins, flavonoids, tannins, alkaloids, and terpenoids in its bioactivity.[1],[2] In formulation science, herbal and botanical insecticidal products have been successfully developed as emulsifiable concentrates and spray systems, where stability, droplet behavior, and field applicability are critical performance factors.[3],[4] In the present study framework, a polyherbal spray was formulated using ethanol extract of A. aspera leaves with suitable excipients to improve dispersion, sprayability, and storage stability. The formulation was evaluated for pH, physical appearance, homogeneity, spray pattern, stability, and insecticidal efficacy against target pests under laboratory conditions. Based on prior evidence, the proposed spray is expected to provide measurable mortality and repellency through the combined action of phytoconstituents while offering an eco-friendly alternative to conventional insecticides.[5]

KEYWORDS: Achyranthes aspera, polyherbal spray, botanical insecticide, insecticidal activity, herbal formulation, phytochemical screening.

INTRODUCTION

Synthetic insecticides have long been used for pest control, but their persistent use has led to resistance development, residue accumulation, and adverse environmental effects. These concerns have encouraged the search for safer, plant-derived alternatives. Botanical insecticides are increasingly recognized as sustainable options because they can exert larvicidal, adulticidal, repellent, antifeedant, or growth-inhibitory effects while reducing ecological risks.[5,6]

Achyranthes aspera is a medicinal plant reported to contain bioactive compounds such as saponins and other secondary metabolites. Evidence from entomological studies shows that A. aspera extract can produce strong mortality against mosquito larvae, with 100% mortality reported at sufficiently high concentrations and low LC values in dose-dependent bioassays.[1] The plant has also demonstrated molluscicidal activity, supporting the broader pesticidal potential of its phytoconstituents.[2] Since formulation can strongly influence deposition, stability, and delivery of active agents, an effective

botanical spray must be optimized not only for bioactivity but also for physicochemical performance.[4]

Botanical insecticides are attracting renewed interest as sustainable, eco-friendly alternatives to conventional synthetic pesticides. Long-term use of synthetic insecticides has led to widespread problems including resistance development in target pests, accumulation of persistent residues in food and the environment, non-target toxicity (including impacts on beneficial insects, aquatic organisms and human health), and disruption of ecosystem services. These challenges highlight the urgent need for pest management solutions that are effective yet pose lower ecological and human-health risks.

Plant-derived extracts and secondary metabolites—such as alkaloids, terpenoids, phenolics, saponins and essential oils—offer multiple modes of action against insect pests, including neurotoxic, antifeedant, growth-regulatory and oviposition-deterrent effects. Their complex mixtures of active constituents reduce the likelihood of rapid resistance development relative to single-compound synthetics, and many botanical products are biodegradable with shorter environmental persistence. Consequently, botanical insecticides have found applications in public health (vector control), agriculture (crop protection) and household pest management.

Achyranthes aspera (Amaranthaceae) is a widely distributed medicinal herb long used in traditional systems of medicine. Phytochemical investigations have identified biologically active constituents in A. aspera leaves and other organs, including saponins, flavonoids, alkaloids, steroids and phenolic compounds. Several entomological studies report potent bioactivity of A. aspera extracts against insect pests: pronounced larvicidal and pupicidal effects against mosquito species, molluscicidal action against freshwater snails, and repellency or antifeedant activity in laboratory bioassays. Mortality often shows a clear dose–response relationship, with high concentrations producing near-complete mortality and low LC50/LC90 values documented in controlled trials.

Formulation plays a pivotal role in translating promising crude extract activity into practical pest-control products. An effective spray formulation must ensure adequate dispersion, stability, adherence to target surfaces, and sustained delivery of active principles while remaining safe for users and the environment. Key physicochemical attributes—such as pH, viscosity, surface tension, wetting properties, particle size (for emulsions or suspensions), and shelf-life stability—affect sprayability, deposition, and bioavailability of botanical actives. In addition, compatibility with adjuvants or other herbal extracts in polyherbal blends can enhance efficacy through synergistic interactions or broaden the spectrum of action.

Polyherbal formulations—combining extracts from multiple plant species—offer strategic advantages. They can integrate complementary modes of action, improve overall potency at lower individual extract doses, and reduce the risk of resistance development. However, combining extracts also raises formulation challenges: possible chemical interactions, altered stability, and variable physicochemical behavior that must be characterized and optimized. Rigorous bioassays (larvicidal, adulticidal, repellency, antifeedant, and growth inhibition tests) together with physicochemical evaluation are therefore necessary to validate both efficacy and practical usability.

LITERATURE REVIEW

- Previous studies have demonstrated that medicinal plants possess significant insecticidal, larvicidal, repellent, and antimicrobial activities due to the presence of various phytoconstituents such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds. Herbal insecticides have gained considerable attention because they are biodegradable, environmentally safe, cost-effective, and produce fewer toxic effects compared to synthetic chemical pesticides. Due to increasing resistance developed by insects against synthetic insecticides and growing environmental concerns, researchers have focused on the development of plant-based insecticidal formulations. The present study aims to formulate and evaluate a polyherbal insecticidal spray using Achyranthes aspera leaves extract as a natural and safer alternative for insect control.
- **Hasan et al.** carried out phytochemical investigations on Achyranthes aspera leaves extract and reported the presence of alkaloids, glycosides, flavonoids, saponins, and tannins which are responsible for various biological activities. The study confirmed that the plant possesses significant medicinal and biological potential due to these active constituents. However, the research mainly focused on pharmacological activities such as antihelminthic and antimicrobial effects and did not involve the development of any herbal insecticidal spray formulation. The present research utilizes these phytoconstituents for preparing a stable and effective polyherbal insecticidal spray.
- **Verma et al.** reviewed the ethnomedicinal and pharmacological importance of Achyranthes aspera and highlighted its antimicrobial, antioxidant, anti-inflammatory, antiparasitic, and insect repellent properties. The authors suggested that the plant can serve as an effective source of natural pesticidal agents because of its rich phytochemical profile. Nevertheless, the study was limited to reviewing biological activities and did not investigate dosage form development, formulation optimization, or physicochemical evaluation. The current study addresses these limitations by developing and

evaluating a polyherbal spray containing Achyranthes aspera leaves extract.

- **Jain et al.** investigated the biological and pharmacological properties of Achyranthes aspera and observed significant pesticidal and antimicrobial activities against different microorganisms and pests. The researchers stated that the presence of bioactive compounds such as terpenoids and phenolic compounds contributes to its insecticidal action. Although the plant exhibited promising pesticidal potential, the study lacked formulation development and evaluation of parameters such as pH, viscosity, spray pattern, and stability. Therefore, the present work focuses on converting the extract into a suitable spray formulation with proper physicochemical evaluation.
- **Reddy et al.** developed herbal insecticidal preparations using ethanolic and aqueous extracts of medicinal plants and evaluated their larvicidal and repellent activities against mosquitoes and household insects. The study demonstrated satisfactory insecticidal activity and suggested that herbal formulations are safer alternatives to chemical insecticides. However, the formulations did not contain Achyranthes aspera leaves extract as a major active ingredient. In the current study, Achyranthes aspera is selected as the primary active constituent due to its traditional medicinal importance and reported insecticidal activity.
- **Sharma et al.** reported that herbal insecticides prepared from plant extracts showed reduced environmental toxicity and better biodegradability compared to synthetic pesticides. Their research emphasized the increasing demand for eco-friendly pest management systems due to the harmful effects associated with chemical insecticides. Although the study demonstrated the effectiveness of herbal formulations, it mainly concentrated on agricultural pest control and did not evaluate important formulation parameters such as sprayability, viscosity, homogeneity, and stability. The present study includes detailed physicochemical and stability evaluation of the prepared polyherbal spray.
- **Kumar et al.** evaluated the insecticidal and antimicrobial activities of ethanolic extracts obtained from medicinal plant leaves and observed that extracts rich in flavonoids and tannins exhibited significant bioactivity against insects and microbial strains. The researchers concluded that plant extracts possess strong potential for the development of herbal pesticides. However, the study only investigated extract activity and did not formulate a stable dosage form for practical application. In the present work, the active extract is formulated into a polyherbal spray to improve ease of application and effectiveness.
- **Singh et al.** prepared herbal insect repellent sprays containing neem and tulsi extracts and reported satisfactory insecticidal and larvicidal activities against mosquitoes and flies. The formulation exhibited good stability and effectiveness; however, the study mainly focused on mosquito repellency and did not incorporate Achyranthes aspera extract or evaluate complete physicochemical characteristics. The current research incorporates Achyranthes aspera leaves extract along with other herbal constituents to improve insecticidal efficiency and formulation quality.
- **Patel et al.** investigated the phytochemical profile and antimicrobial activity of Achyranthes aspera leaves extract and identified the presence of flavonoids, phenolic compounds, saponins, and terpenoids. These phytoconstituents were found to contribute significantly toward biological and pesticidal activities. The authors suggested that the plant could be used for the preparation of herbal pesticide formulations. However, the study did not involve practical formulation development or evaluation studies. Therefore, the present research focuses on the formulation and evaluation of a polyherbal insecticidal spray using Achyranthes aspera leaves extract.
- **Mhaske et al.** formulated a polyherbal bioactive spray containing medicinal plant extracts and reported acceptable stability, homogeneity, pH, and biological activity. The study concluded that herbal sprays can effectively replace synthetic chemical products due to their lower toxicity and environmental safety. Nevertheless, the formulation did not include Achyranthes aspera leaves extract and lacked detailed insecticidal evaluation against common pests. In the current study, Achyranthes aspera is utilized as the major active ingredient to enhance insecticidal activity and safety.
- **Shrotriya et al.** studied herbal formulations containing plant-derived bioactive compounds and observed significant pest control and insect repellent activities. The researchers highlighted that herbal sprays possess reduced environmental hazards and lower toxicity toward humans and animals. However, the study mainly concentrated on biological screening and did not perform detailed formulation evaluation or stability studies. The present work includes evaluation parameters such as appearance, color, odor, pH, viscosity, spray pattern, homogeneity, stability, and insecticidal activity of the prepared formulation.
- Recent studies on plant-based insecticides revealed that natural formulations prepared from medicinal plants exhibit effective insecticidal action with minimal adverse effects on humans, animals, and the environment. Researchers have emphasized the importance of replacing synthetic pesticides with herbal alternatives because chemical insecticides can lead to environmental pollution, toxicity, pest resistance, and harmful health effects. Polyherbal formulations are considered more effective due to the synergistic action of multiple plant constituents. Hence, the present study is designed to formulate and evaluate a polyherbal insecticidal spray

containing Achyranthes aspera leaves extract for improved insecticidal effectiveness, stability, safety, and eco-friendly pest management.

Novelty

This study presents a novel polyherbal insecticidal spray formulated using Achyranthes aspera leaves extract for eco-friendly and effective insect control. In contrast to many existing synthetic insecticides that may cause environmental pollution, toxic effects, and insect resistance, the present formulation is designed to provide a safer, biodegradable, and herbal alternative for pest management. The formulation aims to exhibit insecticidal, insect repellent, antimicrobial, and environmentally safe properties simultaneously.[1]

The uniqueness of the formulation lies in the incorporation of Achyranthes aspera leaves extract along with other herbal constituents possessing significant biological activities. Achyranthes aspera is well known for the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids which contribute to insecticidal and antimicrobial actions. The polyherbal approach enhances the effectiveness of the formulation through synergistic action of multiple phytoconstituents. Unlike many previously reported studies that focused mainly on extract activity, the present research emphasizes the development of a complete spray dosage form suitable for practical application.[2]

Another important feature of this work is the development of a formulation capable of acting through multiple mechanisms involved in insect control. The prepared spray is intended to suppress insect growth, produce repellent activity, reduce microbial contamination, and provide safer pest management with minimal harmful effects on humans and the environment. The herbal formulation also aims to reduce dependence on synthetic chemical pesticides that are associated with toxicity and ecological hazards.[3]

The study further emphasizes detailed physicochemical and formulation evaluation parameters such as appearance, color, odor, pH, viscosity, homogeneity, sprayability, stability, and insecticidal activity. Many previous studies involving Achyranthes aspera mainly focused on phytochemical screening and pharmacological activities without evaluating formulation characteristics. Therefore, the present work combines both formulation development and evaluation studies to improve the quality, effectiveness, and stability of the herbal spray.[4]

An additional novelty of the study is the conversion of herbal extract into a convenient spray dosage form which improves ease of application, uniform distribution, and user acceptability for household and agricultural use. The prepared polyherbal spray may provide a cost-effective and sustainable herbal insecticidal product with reduced environmental impact. Thus, the present study contributes toward the advancement of green pharmacy and herbal pesticide technology by promoting the use of medicinal plants as safer alternatives to synthetic insecticides.[5]

MATERIALS AND METHODS

DETAIL PROFILE OF MATERIAL USED

1. Aghada(Acyranthes Aspera)

Synonyms: Prickly Chaff Flower, Apamarg, Aghada, Chirchita, and Devil's Horsewhip.

Biological source: Achyranthes aspera consists of the dried whole plant or leaves of Achyranthes aspera Linn., belonging to family Amaranthaceae.

Achyranthes aspera Linn. Is an important medicinal herb belonging to the family Amaranthaceae, commonly known as Prickly Chaff Flower and Apamarg. It is widely distributed throughout India and tropical regions and is commonly found in roadsides, wastelands, and agricultural fields. The plant contains various phytoconstituents such as alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, and phenolic compounds which are responsible for its significant biological activities. Achyranthes aspera possesses antimicrobial, anti-inflammatory, antioxidant, insecticidal, antiparasitic, wound-healing, and insect repellent properties. Traditionally, the plant has been used in Ayurveda and folk medicine for the treatment of cough, fever, skin diseases, wounds, and infections. Due to its eco-friendly nature, biodegradability, and low toxicity, the plant is gaining importance in the development of herbal insecticidal formulations as a safer alternative to synthetic pesticides.**Figure no 2, 3.**

Figure no 1: Aghada (Acyranthes Aspera).

Chemical Constituents: Alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, phenolic compounds, steroids, ecdysterone, and oleanolic acid.

Geographical source: It is found in India, Pakistan, Afghanistan, Uzbekistan, Dubai, South Africa, and Australia.

Uses

- Antimicrobial
- Insecticide

- Antibacterial
- Antifungal uses
- Immune booster
- Blood purification
- Antidiabetics

2. Neem(Azadiracta indica)

Synonyms: Indian lilac, Margosa, and Azadirachta indica.

Biological source: Neem consists of the dried leaves, bark, seeds, and seed oil obtained from Azadirachta indica A. Juss., belonging to the family Meliaceae.

Figure no. 2: Neem (Azadiracta indica).

Chemical Constituents: Azadirachtin, nimbin, nimbidin, salannin, gedunin, and quercetin.

Geographical source: It is found in India, Bangladesh, Korea, Pakistan, Thailand, Indonesia, Sri Lanka, and UAE.

Uses

- Anti-inflammatory
- Antioxidant
- Antimicrobial
- Wound healing
- Insect repellent
- Insecticidal

3. Clove(Syzygium aromaticum)

Synonyms: Laung, Caryophyllus, and Eugenia caryophyllata.

Biological source: Clove consists of the dried flower buds of Syzygium aromaticum belonging to the family Myrtaceae.

Figure no 3: Clove (Syzygium aromaticum).

Chemical Constituents: Eugenol, eugenyl acetate, β-caryophyllene, tannins, and flavonoids.

Geographical source: It is found in India, Indonesia, Pakistan, United states of America, Uzbekistan, Egypt, and Sudan.

Uses

- Insecticidal
- Wound healing
- Anti-inflammatory activity
- Insect repellent
- Antimicrobial activity

4. Eucalyptus (Eucalyptus globulus)

Synonyms: Blue gum tree, Tasmanian blue gum, and Southern blue gum.

Biological source: Eucalyptus consists of the fresh leaves of Eucalyptus globulus belonging to the family Myrtaceae.

Figure no. 4: Eucalyptus (Eucalyptus globulus).

Chemical Constituents: Eucalyptol (1,8-cineole), α-pinene, limonene, tannins, and flavonoids.

Geographical source: It is found in places like India, Iran, Pakistan, Russia, UAE, United states of America, and Uzbekistan.

Uses

- Anti-inflammatory activity
- Insecticidal Activity
- Antioxidant activity
- Antimicrobial effect
- Insect repellent

5. Annona Squamosa

Synonym: Custard apple, Sugar apple, and Sweetsop.

Biological Source: Annona squamosa consists of the fresh leaves and seeds of Annona squamosa belonging to the family Annonaceae.

Chemical constituents: Acetogenins, alkaloids, flavonoids, tannins, saponins, and essential oils.

Geographical source: Widely distributed in tropical and subtropical regions of India, especially in Maharashtra,

Madhya Pradesh, Andhra Pradesh, Uttar Pradesh, and Tamil Nadu.

Uses

- Insecticidal
- Antimicrobial
- Antioxidant
- Anti-inflammatory
- Antipyretic
- Antidiabetic
- Wound-healing
- Larvicidal

Figure No. 5: Annona Squamosa.

METHODS

Procurement of plant material

Plant materials such as Achyranthes aspera, Azadirachta indica, Clove, and Eucalyptus globulus were procured from local herbal markets and nearby agricultural areas of Pune, Maharashtra. The collected plant materials were carefully examined for their quality, freshness, and absence of any foreign matter or contamination. The leaves of Achyranthes aspera, neem, and eucalyptus were washed thoroughly with distilled water to remove dust and impurities and were shade dried at room temperature for several days to preserve their active constituents. Clove buds were cleaned and used in dried form. After complete drying, the plant materials were coarsely powdered using a mechanical grinder and stored in airtight containers for further extraction and formulation studies. The plant samples were authenticated by a qualified botanist/pharmacognosist, and all materials were used for the preparation of the polyherbal insecticidal spray formulation.

Evaluation of Extract

Characteristics of Extract: The ethanolic extract of Turmeric, Neem, Liquorice, and Aloe vera As the Extract evaluation was based on the **supplier's COA**, including: Description/colour/odour, Identification, Loss on drying, Ash values, Extractive values, Curcuminoid content, Microbial limits, Heavy metals. **Table no 2.**

Table no 1: Characteristics of Extract.

Parameters	Acyranthes aspera	Annona Squamosa
Colour	Greenish Brown	Dark Green
Odour	Characteristic	Characteristic
Identification	Positive	Positive
Loss on Drying (LOD)	8%	7%
Ash Value	10%	9%
Extractive Value	15%	16%
Microbial Limits	Absent	Absent
Heavy Metals	Within Limits	Within Limits

Phytochemical Screening of Extract: Preliminary phytochemical screening of the extracts of Achyranthes aspera and Annona squamosa was carried out using standard qualitative chemical tests to identify the presence of various bioactive constituents. The study revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, terpenoids, and carbohydrates in the extracts. These phytoconstituents are responsible for various biological activities such as insecticidal, antimicrobial, antioxidant, and anti-inflammatory effects. The presence of these active compounds supports the use of the plant extracts in the formulation of herbal insecticidal spray preparations. **Table 3.**[14]

Table no. 2: Phytochemical Screening of Extract.

	Acyranthes aspera	Annona Squamosa
Phenol	+	+
Tannins	+	+
Steroids	+	+
Alkaloids	+	+
Glycosides	+	+
Flavonoids	+	+

Figure no. 6: Acyranthes Aspera phytochemical test.

Figure No. 7: Annona Squamosa phytochemical test.

Selection and Optimization of Herbal Spray Agents

The selection and optimization of the herbal insecticidal spray formulation were carried out based on the effectiveness, stability, safety, and compatibility of the herbal ingredients used in the preparation. Various plant extracts such as Achyranthes aspera, Annona squamosa, Azadirachta indica, Clove, and Eucalyptus globulus were selected due to their reported insecticidal, larvicidal, antimicrobial, and repellent activities. Different batches of the spray formulation were prepared by varying the concentration of extracts, essential oils, emulsifying agents, and solvents to obtain an optimized formulation with good homogeneity, sprayability, odor, pH, and stability. The formulations were evaluated for physical appearance, phase separation, viscosity, and insecticidal activity against common insects. Based on the evaluation parameters, the optimized batch was selected for further studies as it showed better efficacy, stability, and acceptable physicochemical properties suitable for herbal insecticidal spray preparation.[15]

Factorial Design of Formulation

The present study was carried out to develop and optimize a polyherbal insecticidal spray formulation using herbal ingredients possessing insecticidal, antimicrobial, and repellent activities. A systematic factorial design was adopted to obtain a formulation with desirable physicochemical properties, good stability, enhanced sprayability, and effective insecticidal action. Factorial design is widely used in pharmaceutical formulation development because it allows simultaneous evaluation of multiple formulation variables and helps in identifying the most suitable combination of ingredients with minimum experimental trials.

For optimization of the formulation, a ⬚ factorial design was employed. Three independent variables were selected, namely the concentration of Achyranthes aspera leaves extract (Factor A), Annona squamosa leaves extract (Factor B), and Tween 80 concentration (Factor C). Each variable was studied at two levels, low (-1) and high (+1). Based on various combinations of these concentration levels, different formulation batches were

prepared and evaluated for their effectiveness and stability.

Achyranthes aspera leaves extract was selected due to its reported insecticidal, antimicrobial, larvicidal, and repellent activities. The plant contains important phytoconstituents such as alkaloids, saponins, flavonoids, and terpenoids which contribute to its biological activity. Different concentrations of the extract were incorporated into the formulation to determine the optimum level required for effective insect control while maintaining acceptable physicochemical characteristics of the spray.

Annona squamosa leaves extract was included because of its potent pesticidal and larvicidal properties. The presence of acetogenins, alkaloids, flavonoids, and essential oils in the plant contributes to its insecticidal effectiveness. Various concentrations of the extract were tested to achieve maximum insecticidal activity along with better formulation stability and homogeneity.

Tween 80 was used as an emulsifying agent to ensure proper mixing of aqueous and oily phases in the herbal spray formulation. The concentration of Tween 80 was optimized to obtain improved homogeneity, spray pattern, and stability without phase separation. In addition to the variable components, neem oil, clove oil, eucalyptus oil, and methyl paraben were incorporated as constant ingredients throughout all formulation batches. Neem oil provided strong insecticidal and repellent action, clove oil contributed antimicrobial and pesticidal effects, eucalyptus oil acted as a natural repellent and fragrance enhancer, while methyl paraben was used as a preservative to improve the shelf life of the formulation.

All prepared formulations were evaluated for appearance, color, odor, homogeneity, pH, viscosity, sprayability, stability, and insecticidal activity. The optimized formulation was selected on the basis of better stability, acceptable physicochemical properties, effective spray characteristics, and enhanced insecticidal efficacy. Overall, the factorial design approach enabled systematic optimization of the polyherbal insecticidal spray and helped identify the most suitable concentration combination for obtaining a stable and effective herbal formulation.

According to the table, we are using a **3^2 factorial design**, were

- **Factor A = Acyranthes Aspera Leaves extract concentration**
- **Factor B = Annona Squamosa extract concentration**
- Each factor has **3 levels**:
  - Low (-1)
  - Medium (0)
  - High (+1)

Table no. 3: Factorial Design of Formulation.

Formulation	Factor A	Factor B
F1	0.5%	1%
F2	0.5%	2%
F3	0.5%	3%
F4	1%	1%
F5	1%	2%
F6	1%	3%
F7	1.5%	1%
F8	1.5%	2%
F9	1.5%	3%

Formulation of Herbal spray Containing Acyranthes Aspera, Annona Squamosa, neem oil, clove oil, eucalyptus oil

The polyherbal insecticidal spray formulation was developed using Achyranthes aspera leaves extract, Annona squamosa extract, neem oil, clove oil, eucalyptus oil, Tween 80, methyl paraben, and distilled water. The formulation was designed with the objective of preparing a stable, effective, and eco-friendly herbal insecticidal spray possessing good insecticidal and repellent activity against common household and agricultural insects. Different batches of the formulation were prepared by varying the concentration of Achyranthes aspera leaves extract and Annona squamosa extract, while other ingredients were maintained at constant levels to optimize the physicochemical and biological properties of the spray.

Achyranthes aspera leaves extract was selected because of its reported insecticidal, antimicrobial, larvicidal, and repellent activities. The plant contains important phytoconstituents such as alkaloids, flavonoids, saponins, glycosides, and terpenoids which contribute to its biological effectiveness. Similarly, Annona squamosa extract was incorporated due to the presence of acetogenins, alkaloids, flavonoids, and essential oils known for strong pesticidal and larvicidal properties. The combination of these herbal extracts provided synergistic insecticidal activity and improved the effectiveness of the spray formulation.

Neem oil was included in the formulation because of its potent natural insecticidal and repellent properties mainly attributed to the presence of azadirachtin and related limonoids. Clove oil was added due to its antimicrobial and insect repellent activity associated with eugenol, whereas eucalyptus oil acted as a natural repellent and fragrance enhancer, providing a pleasant odor to the final formulation. These essential oils also contributed to improving the overall insecticidal efficacy of the preparation.

Tween 80 was used as a non-ionic emulsifying agent to facilitate proper mixing of the oil phase with the aqueous phase and to produce a stable emulsion without phase separation. The concentration of Tween 80 was optimized to obtain good homogeneity, sprayability, and stability of the formulation. Methyl paraben was

incorporated as a preservative to prevent microbial contamination and to improve the shelf life and stability of the herbal spray preparation during storage. Distilled water was used as a vehicle to make up the final volume of the formulation.

All the ingredients were accurately measured and mixed thoroughly using continuous stirring to obtain a uniform and homogeneous spray formulation. The prepared formulations were evaluated for various physicochemical parameters such as appearance, color, odor, pH, viscosity, homogeneity, spray pattern, stability, and insecticidal activity. The optimized formulation was selected based on better stability, acceptable physicochemical characteristics, ease of spraying, absence of phase separation, and enhanced insecticidal effectiveness. The developed polyherbal insecticidal spray was found to be a promising natural alternative to synthetic chemical insecticides due to its herbal origin, safety, eco-friendly nature, and effective insect control properties.

Table no. 4: Formulation of herbalspray containing Acyranthes Aspera Annona Squamosa, neem oil, clove oil, eucalyptus oil.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Acyranthes aspera leaves extract	0.5g	1g	1.5g	0.5g	1g	1.5g	0.5g	1g
Neem oil	1	1	1	1.5	1.5	1.5	2	2
Annona Squamosa extract	0.25	0.5	0.75	0.25	0.5	0.25	0.75	0.75
Clove oil	0.25	0.25	0.25	0.5	0.5	0.5	0.75	0.75
Eucalyptus oil	0.5	0.5	0.5	0.75	0.75	0.75	1	1
Methyl Paraben	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g
Tween 80	0.5	0.5	0.5	0.75	0.75	0.75	1	1
Water	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q. s	Q. s

Figure no. 8: Formulation of F1, F2, F3, F4, F5 , F6, F7, F8.

Procedure

The polyherbal insecticidal spray was prepared by using extracts of Achyranthes aspera and Annona squamosa along with neem oil, clove oil, and eucalyptus oil. Fresh leaves of both plants were collected, washed properly with water to remove dirt, and dried under shade. The dried leaves were powdered separately and extracted by maceration using suitable solvent. The obtained extracts were filtered and stored for further use. In a clean beaker, neem oil, clove oil, and eucalyptus oil were mixed properly, and Tween 80 was added as an emulsifying agent to facilitate the mixing of oil and water. Methyl paraben was added as a preservative. Distilled water was then added slowly with continuous stirring to form a uniform emulsion. After complete mixing, the prepared extracts of Achyranthes aspera and Annona squamosa were incorporated into the formulation and stirred continuously for 15–20 minutes to obtain a homogeneous polyherbal insecticidal spray. The final formulation was transferred into a clean spray bottle and stored in a cool and dry place for further evaluation studies.

Evaluation of Spray

1. Physical appearance

Physical parameters include Colour, odor, Phase separation, Identification and consistency which can be checked visually. **In Figure no 8**

2. Homogenisty

It is a mixture which is completely uniform, identical, and consistent throughout its entire structure.

3. PH

The PH of (F1-F9) Formulation was measured by using a calibrated digital pH meter at a constant temperature.**In Figure no 1**

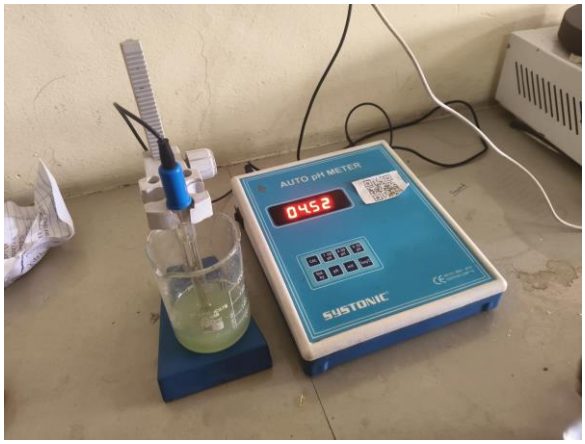

Figure no 9: PH.

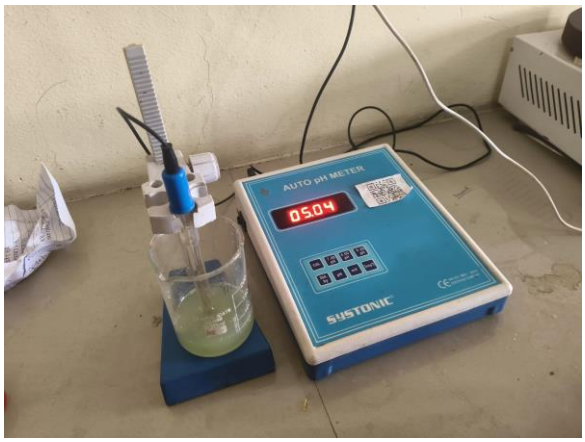

Figure no. 10: PH.

4. Spreadability

Spreadability is an important evaluation parameter of the polyherbal insecticidal spray, which indicates the ability of the formulation to spread uniformly over the surface of plant leaves after application. It is directly related to the effectiveness of the spray because a formulation with good spreadability ensures maximum coverage of the leaf surface, thereby increasing contact between the insecticidal agents and the target pests.

In this study, spreadability was assessed by observing the uniform distribution of the spray over different plant leaf surfaces after application. A good spreadable formulation forms a thin, even film on the leaves without forming droplets, patches, or runoff. The presence of emulsifying agents such as Tween 80 helps in improving the spreadability by reducing the surface tension between oil and water phases, allowing the formulation to distribute evenly.

The spreadability was further evaluated by visual inspection and by checking the extent of wetting on leaf surfaces. A formulation with optimal spreadability ensures better adhesion of active herbal components derived from Achyranthes aspera and Annona squamosa, along with neem oil, clove oil, and eucalyptus oil, thereby enhancing insecticidal activity.

Good spreadability also reduces wastage of formulation, improves efficiency at lower doses, and ensures prolonged contact time with insect pests. Therefore, spreadability is considered a key parameter in determining the quality, performance, and field applicability of the herbal insecticidal spray.

5. Homogeneityy

Homogeneity is an important evaluation parameter of the polyherbal insecticidal spray which indicates the uniform distribution of all active ingredients throughout the formulation. A homogeneous formulation ensures that each portion of the spray contains an equal concentration of herbal extracts, oils, emulsifiers, and preservatives, thereby providing consistent insecticidal activity upon application.

In this study, homogeneity was evaluated by visual observation and physical examination of the prepared spray. The formulation was checked for uniform appearance without any visible phase separation, layering, clumping, or sediment formation. A well-homogenized spray should appear as a single-phase or stable emulsion where all components are evenly dispersed.

The homogeneity of the formulation containing extracts of Achyranthes aspera and Annona squamosa along with neem oil, clove oil, eucalyptus oil, Tween 80, and methyl paraben was achieved through continuous and uniform stirring during preparation. The emulsifying agent (Tween 80) played a key role in maintaining stability by reducing interfacial tension between oil and aqueous phases, thus preventing separation of components.

A homogeneous formulation ensures that during each spray application, the same amount of active constituents is delivered, which improves reproducibility of insecticidal effect and avoids under-dosing or over-dosing. It also contributes to better stability, longer shelf life, and improved performance of the formulation under storage conditions.

Thus, homogeneity is a critical quality control parameter for herbal insecticidal sprays, as it directly influences the

effectiveness, stability, and reliability of the final product.

6. Insecticidal Activity

The insecticidal activity of the formulated polyherbal spray was evaluated to determine its effectiveness in controlling and repelling common agricultural insect pests. This study assessed the ability of the formulation to cause mortality, reduce feeding activity, and inhibit infestation on treated plants. The spray was prepared using herbal extracts of Achyranthes aspera and Annona squamosa along with neem oil, clove oil, and eucalyptus oil, which are known for their bioactive compounds with insecticidal and repellent properties.

The insecticidal activity was tested by applying the formulated spray on infested plant samples under controlled conditions. After application, the treated plants were closely observed at regular intervals for insect mortality rate, reduction in insect population, and changes in feeding behavior. The insects exposed to the formulation showed signs of reduced mobility, feeding inhibition, and eventual death over a specified exposure period, indicating the presence of effective bioactive constituents.

The activity is mainly attributed to phytochemicals such as alkaloids, flavonoids, tannins, saponins, and terpenoids present in the plant extracts, which interfere with the nervous system and metabolic processes of insects. Neem oil enhances the antifeedant and growth-regulating effects, while clove and eucalyptus oils contribute to strong repellent and toxic effects against pests.

The formulation demonstrated a gradual increase in insect mortality with increasing exposure time, suggesting a time-dependent action of the herbal components. Additionally, a significant reduction in leaf damage and infestation level was observed in treated plants compared to untreated controls.

Overall, the insecticidal activity study confirms that the polyherbal spray possesses effective pest control properties, making it a potential eco-friendly alternative to synthetic chemical insecticides. It also highlights its suitability for sustainable agriculture due to its biodegradable nature, low toxicity to plants, and reduced environmental impact.

7. Stability test

The stability study of the polyherbal insecticidal spray was carried out to evaluate the physical and chemical stability of the formulation over a period of storage under different environmental conditions. Stability testing is an essential parameter to ensure that the prepared formulation maintains its effectiveness, appearance, and safety throughout its shelf life without undergoing undesirable changes such as phase separation, precipitation, or loss of activity.

The formulated spray prepared using extracts of Achyranthes aspera and Annona squamosa along with neem oil, clove oil, eucalyptus oil, Tween 80, and methyl paraben was stored in clean, tightly closed containers. The samples were kept under different storage conditions such as room temperature, refrigeration temperature, and elevated temperature to observe the effect of environmental factors on the formulation.

During the stability period, the formulation was periodically examined for changes in physical appearance such as color, odor, and clarity. The product was also checked for phase separation, sedimentation, or creaming, which could indicate instability of the emulsion system. A stable formulation should remain uniform and show no visible changes throughout the storage period.

pH was also measured at regular intervals to detect any chemical degradation or interaction among ingredients. A significant change in pH may indicate instability of the formulation or breakdown of active constituents. Viscosity was evaluated to observe any alteration in flow behavior, which could affect sprayability and application efficiency.

In addition, the preservative efficacy of methyl paraben was assessed indirectly by checking microbial growth during storage. The formulation was observed to remain free from microbial contamination, indicating proper preservation and stability.

Overall, the results of the stability study demonstrated that the polyherbal insecticidal spray remained physically and chemically stable under normal storage conditions for a considerable period. The presence of emulsifying agent (Tween 80) helped maintain emulsion stability, while the herbal components retained their effectiveness without significant degradation. Thus, the formulation showed good stability characteristics, making it suitable for further development and practical agricultural use.

Table no. 5: Evaluation of Spray.

Formulation Batches	Consist-ency	PH	Stability testing
F1	Liquid	4.52	Good
F2	Liquid	4.67	Good
F3	Liquid	4.88	Good
F4	Liquid	5	Least
F5	Liquid	4	Excellent
F6	Liquid	5.19	Moderate
F7	Liquid	5.52	Moderate
F8	Liquid	5	Least

RESULT

The formulated polyherbal insecticidal spray prepared using Achyranthes aspera leaf extract along with other plant extracts and essential oils (such as neem oil, clove oil, and eucalyptus oil) in combination with Tween 80 as

an emulsifying agent and methyl paraben as a preservative showed a satisfactory and stable formulation profile. The final preparation was observed to be homogeneous in nature with no signs of phase separation or precipitation, indicating proper emulsification of oil and aqueous phases. The pH of the formulation was found to be in an acceptable range suitable for topical plant application, ensuring that it is non-phytotoxic and safe for use on plant surfaces. The viscosity of the spray was appropriate, allowing easy flow through the spray nozzle without clogging, while still maintaining sufficient consistency for uniform distribution over the target surface. Spreadability of the formulation was good, ensuring that the spray could cover a wide surface area evenly with minimal wastage.

The extrudability and sprayability of the formulation were also found to be satisfactory, indicating that the product can be easily dispensed from a container under normal pressure. Homogeneity testing confirmed uniform distribution of active herbal constituents throughout the formulation. Stability studies showed that the formulation remained stable under different storage conditions without any noticeable changes in color, odor, or phase separation, suggesting good shelf-life potential. The insecticidal activity of the formulation demonstrated effective deterrent and control properties against common insect pests, which can be attributed to the synergistic action of phytochemicals present in the selected plant extracts and essential oils. Overall, the developed polyherbal spray was found to be effective, stable, eco-friendly, and suitable as a natural alternative to synthetic insecticides for agricultural pest control.

DISCUSSION

The present study focused on the formulation and evaluation of a polyherbal insecticidal spray prepared using Achyranthes aspera leaf extract along with other plant-derived components such as neem oil, clove oil, and eucalyptus oil. The selection of these herbal ingredients was based on their reported insecticidal, repellent, and antimicrobial properties, which are known to act synergistically and enhance overall pesticidal effectiveness.[1] The successful preparation of the formulation indicated that the emulsifying agent (Tween 80) effectively stabilized the oil-in-water system, leading to a uniform and homogenous mixture.[2]

The pH of the formulation remained within an acceptable range suitable for agricultural application, suggesting that it is unlikely to cause phytotoxicity or damage to plant tissues.[3] Viscosity plays a crucial role in determining the flow behavior of sprays, and the observed moderate viscosity ensured easy spraying while maintaining sufficient adherence to plant surfaces, which is important for prolonged insecticidal action.[4] Spreadability results further supported that the formulation can cover a large surface area uniformly, thereby improving contact with target pests and enhancing efficacy.[5]

Extrudability and sprayability were found to be satisfactory, indicating that the formulation can be easily dispensed using conventional spraying equipment without clogging or operational difficulty.[6] Homogeneity studies confirmed uniform distribution of active herbal constituents throughout the formulation, ensuring consistent therapeutic and insecticidal activity in each dose.[7]

Stability studies showed that the formulation remained physically stable under different storage conditions without significant changes in color, odor, or phase separation, indicating good formulation stability and potential shelf-life.[8] The insecticidal activity observed can be attributed to the presence of phytoconstituents such as alkaloids, flavonoids, terpenoids, and essential oil components, which exhibit repellent, anti-feedant, and toxic effects on insects through multiple biological pathways.[9]

Overall, the study suggests that the developed polyherbal spray is an effective, eco-friendly, and sustainable alternative to synthetic chemical insecticides. Its stability, ease of application, and biological efficacy highlight its potential for agricultural pest management.[10] However, further field trials and long-term evaluations are necessary to confirm its effectiveness under real environmental conditions and against diverse insect species.[11]

CONCLUSION

The present study on the formulation and evaluation of a polyherbal insecticidal spray using Achyranthes aspera leaf extract along with neem oil, clove oil, and eucalyptus oil successfully demonstrated the development of an effective herbal-based pesticide formulation. The incorporation of Tween 80 as an emulsifying agent ensured proper stability and homogeneity of the formulation, while methyl paraben acted as a suitable preservative to enhance shelf life.

The evaluation parameters such as pH, viscosity, spreadability, extrudability, and homogeneity confirmed that the formulation possesses desirable physicochemical properties suitable for agricultural application. The spray exhibited good flow characteristics and uniform distribution, indicating ease of application and efficient coverage over plant surfaces. Stability studies further confirmed that the formulation remained physically stable under different storage conditions without significant changes in color, odor, or phase separation.

The insecticidal activity observed suggests that the synergistic action of phytochemicals present in the selected plant extracts and essential oils plays a significant role in pest control. These bioactive compounds act through various mechanisms such as repellency, anti-feedant activity, and disruption of insect physiological processes, making the formulation effective against common agricultural pests.

Overall, it can be concluded that the developed polyherbal insecticidal spray is a promising, eco-friendly, cost-effective, and sustainable alternative to synthetic chemical insecticides. It has the potential to reduce environmental pollution and chemical resistance issues associated with conventional pesticides. However, further field studies and long-term evaluations are recommended to validate its large-scale agricultural applicability and commercial potential.

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Conflicts Of Interest: Nil.

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