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Research on Influence of Formulation Type on Stability and Acute Toxicity of Fluazinam Formulations

Vu Cong Duy[1], Vu Van Vu[2], Hoang Thi Lien[3], Le Thi Thuy[1,*]
[1]School of Chemistry and Life Sciences, Hanoi University of Science and Technology, Ha Noi, Vietnam [2]Northern Pesticides Control and Testing Centre, Ha Noi, Vietnam
[3]Hitech High Quality Control Joint Stock Company
[*]Corresponding author email: thuy.lethi@hust.edu.vn

Abstract

Fluazinam is a contact fungicide widely used for the control of various plant diseases due to its broad spectrum of activity and multi-site mode of action. However, its extremely low water solubility and potential environmental toxicity require careful formulation design to ensure effective performance and safety during practical application. In this study, fluazinam was formulated into three different formulation types, including suspension concentrate (SC), wettable powder (WP), and water-dispersible granule (WDG), at laboratory scale. Selected co-formulants were applied to improve formulation performance and stability. The prepared formulations were evaluated for physicochemical stability, including active ingredient content and suspensibility, under room temperature and accelerated storage conditions. Acute toxicity was assessed according to OECD (Organization for Economic Cooperation and Development) guidelines and classified based on GHS (Globally Harmonized System of Classification and Labelling of Chemicals) 2019 criteria. Although all formulations exhibited low acute toxicity, formulation-dependent differences in toxicological responses were observed. In particular, the SC formulation showed milder and more rapidly reversible effects in inhalation and irritation studies. These results demonstrate that formulation design influences practical exposure characteristics and toxicological behavior beyond the intrinsic properties of the active ingredient.

Keywords: Fluazinam, formulation type, stability, suspension concentrate, toxicity, wettable powder, water-dispersible granule.

1. Introduction

Fungicides play an essential role in modern agriculture by protecting crops from fungal diseases that can cause significant yield losses. Among them, fluazinam is a non-systemic contact fungicide characterized by a broad spectrum of activity and a multi-site mode of action, which contributes to a low risk of resistance development [1, 2]. Owing to these advantages, fluazinam has been widely used for the control of various fungal diseases on economically important crops such as potatoes, vegetables, fruits, and ornamental plants.

From a chemical perspective, fluazinam is a highly lipophilic compound with extremely low water solubility. These physicochemical properties impose significant challenges in formulation development, particularly in achieving homogeneous dispersion in aqueous spray solutions, stable storage behavior, and effective coverage on plant surfaces [3, 4]. In addition, fluazinam has been reported to exhibit high toxicity toward aquatic organisms, which raises concerns regarding environmental exposure and necessitates careful risk management during formulation design and application [5].

In practical pesticide use, the toxicological and exposure characteristics of a product are not solely determined by the intrinsic properties of the active ingredient but are also strongly influenced by formulation-related factors. Components such as solvents, surfactants, dispersing agents, and particle size modifiers play a critical role in determining dispersion behavior, aerosol formation, dustiness, and operator exposure during handling and application [6, 7]. Consequently, evaluation of pesticide safety at the formulation level has become an increasingly important aspect of sustainable agrochemical development [8].

Fluazinam has been formulated into several conventional formulation types, including wettable powder (WP), suspension concentrate (SC), and water-dispersible granule (WDG), each exhibiting distinct advantages and limitations [9]. WP formulations are relatively simple to manufacture and have been widely used for decades; however, they are often associated with dust formation during handling

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and mixing, which may increase the risk of inhalation exposure to operators. SC formulations, in contrast, consist of finely dispersed solid particles suspended in an aqueous medium, offering reduced dustiness, improved handling safety, and better control of particle size distribution. WDG formulations were developed to combine the advantages of solid formulations with improved handling properties, although dust generation may still occur during granule disintegration and dispersion.

Previous studies have demonstrated that formulation design can significantly influence the toxicological behavior of pesticide products, particularly with respect to inhalation exposure and irritation potential. Suspension concentrates have often been reported to exhibit lower inhalation hazards compared with powder-based formulations, primarily due to reduced dust formation and more controlled aerosol generation during application. Nevertheless, such observations are often reported across different active ingredients, and systematic comparative studies focusing on a single active ingredient formulated into multiple formulation types under identical experimental conditions remain limited.

For fluazinam in particular, existing literature has primarily focused on its fungicidal efficacy, residue behavior, and environmental risk assessment. While general formulation guidelines for fluazinam are available, comparative evaluations addressing how different formulation types affect physicochemical stability and toxicological responses of fluazinam-based products are still scarce. This lack of formulation-level comparative data represents an important research gap, especially in the context of developing safer and more sustainable pesticide products.

In this context, the present study aims to formulate fluazinam into three commonly used formulation types, SC, WP, and WDG at laboratory scale and to evaluate formulation-dependent differences in physicochemical stability and acute toxicity. Particular emphasis is placed on key stability indicators, including active ingredient content and suspensibility, as well as acute toxicity endpoints assessed according to OECD guidelines and classified based on GHS criteria. By comparing multiple formulation types under standardized conditions, this study seeks to provide scientific evidence supporting the selection of formulation strategies that improve product stability while minimizing exposure-related toxicological risks.

2. Materials and Methods

2.1. Materials

The formulations shown in Table 1 were selected based on preliminary laboratory optimization studies and subsequently used for stability and acute toxicity evaluations.

Fluazinam (purity 98%) was supplied by Shandong Jihui Chemical Co., Ltd. (China) and used as the active ingredient without further purification. All co-formulants employed in this study were commercial-grade materials commonly used in pesticide formulation and were selected based on their functional roles and compatibility with fluazinam.

Table 1. Composition of fluazinam formulations used in this study

Component	Function	SC (%w/w)	WP (%w/w)	WG (%w/w)
Fluazinam technical (98%)	Active ingredient	50.0	50.0	50.0
7227-A	Dispersant/surfactant	7.0	-	-
Citric acid	pH regulator	1.0	-	-
Propylene glycol	Antifreeze agent	5.0	-	-
Hencide PG520	Preservative	0.2	-	-
AF1501	Defoamer	0.2	-	-
Xanthan gum	Rheology modifier	0.1	-	-
WP-2	Dispersing agent	-	15.0	-
Silica white	Anti-caking agent	-	2.0	-
D350	Dispersing agent	-	-	5.0
D252	Dispersing agent	-	-	5.0
DW12	Wetting agent	-	-	2.0
Corn starch	Binder	-	-	5.0
Ammonium sulfate	Disintegrating agent	-	-	5.0
Kaolin	Carrier	-	q.s.	q.s.
Water RO	Dispersion medium	q.s.	-	-

For WP formulations, dispersing agents, wetting agents, anti-caking agents, and inert carriers were used to ensure adequate wettability, dispersion, and flowability of the powder during handling and application. WDG formulations additionally incorporated binders and disintegrants to facilitate granule formation and rapid disintegration upon contact with water. SC formulations consisted of surfactants, pH regulators, antifreeze agents, preservatives, defoamers, and rheology modifiers dispersed in an aqueous continuous phase to obtain a physically stable suspension [10].

All reagents and auxiliary materials were selected in accordance with general formulation guidelines for agrochemical products and are representative of materials commonly available for industrial formulation practice.

2.2. Preparation of Fluazinam Formulations

2.2.1. Suspension concentrate

The composition of the SC formulation is presented in Table 1. SC formulations were prepared at laboratory scale using a combination of high-speed dispersion and vertical ball milling, which is a commonly applied technique for producing stable suspension concentrates with controlled particle size distributions [11]. Initially, fluazinam and selected co-formulants were dispersed in the aqueous phase using high-speed mechanical stirring to obtain a homogeneous premix. This step promotes initial wetting of solid particles and prevents agglomeration prior to milling [12].

The premix was subsequently subjected to vertical ball milling at 4000 rpm for approximately 1 hour while maintaining the temperature below 10 ºC (using a cooling-water circulation system) to reduce particle size and achieve uniform dispersion of fluazinam particles within the suspension. Following milling, an additional homogenization step of 1 hour was applied to further improve suspension uniformity and stability. Milling and homogenization conditions were optimized to obtain a median particle size D50 smaller or equal 5 µm and a maximum particle size D98 smaller or equal 10 µm, which are generally considered suitable to ensure good suspensibility and spray performance of SC formulations.

2.2.2. Wettable powder

The composition of the WP formulation is presented in Table 1. WP formulations were prepared by dry blending fluazinam with selected co-formulants, followed by particle size reduction using jet milling. Jet milling is widely used in the preparation of powder-based pesticide formulations due to its ability to produce fine particles without excessive thermal stress.

The blended mixture was micronized using a jet mill operated with dry compressed air at 0.6–0.8 MPa. The milling conditions were adjusted to obtain a fine powder with a particle size distribution characterized by D98 smaller or equal 25 µm. This particle size range is commonly applied in WP formulations to ensure adequate wettability, dispersion, and suspensibility in water while minimizing excessive dust generation.

2.2.3. Water-dispersible granule

The composition of the WDG formulation is presented in Table 1. WDG formulations were prepared by granulation of premixed powders followed by hot-air drying. In this process, fluazinam and selected co-formulants were first dry-mixed to obtain a uniform powder blend. Granulation was then carried out using a compaction-based approach to form granules of controlled size and mechanical strength.

Approximately 10% water was added to the dry blend before granulation. Cylindrical granules of approximately 1 mm diameter were produced. The granules were subsequently dried using hot air at temperatures below 60 ºC until the moisture content reached 2–3%, thereby improving storage stability. The WDG formulation approach aims to combine the handling advantages of solid formulations with improved dispersion behavior compared to conventional powders, although disintegration and dispersion performance remain dependent on granule structure and formulation composition.

2.3. Stability Evaluation

The prepared formulations were evaluated for physicochemical stability under various storage conditions to assess their suitability for practical use. All samples were stored at room temperature from May 2025 until evaluation. Accelerated storage tests were conducted at 54 °C for 14 days, which is a commonly applied condition to simulate long-term storage behavior of pesticide formulations. In addition, SC formulations were subjected to low-temperature storage at 0 °C for 7 days to assess freeze–thaw stability and resistance to phase separation [13].

Stability evaluation included both chemical and physical stability parameters. Chemical stability was evaluated by determining fluazinam content using high-performance liquid chromatography (HPLC) with ultraviolet detection according to TCCS 319:2015/BVTV issued by the Plant Protection Department of Vietnam. Analyses were performed on an Agilent 1260 Infinity II HPLC/DAD system equipped with a GL Sciences C18 column (150 mm × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and water (pH 3.0, adjusted with phosphoric acid containing 0.02% triethylamine) at a ratio of 50:50 (v/v). The flow rate was 1.0 mL/min, the

detection wavelength was 240 nm, and the injection volume was 5 µL. Fluazinam content was quantified by comparison of sample and reference standard peak areas.

Physical stability was evaluated using formulation-specific indicators, including appearance, phase separation, suspensibility, wettability (for WP and WDG), and pH variation.

Active ingredient content and suspensibility were considered key indicators of formulation stability, as they directly affect product quality, field performance, and user safety. These parameters were evaluated before and after storage to identify any significant changes attributable to formulation type or storage conditions.

2.4. Toxicity Studies

Acute toxicity and irritation studies were conducted in accordance with the relevant OECD guidelines, including acute oral toxicity (OECD 423/425), acute dermal toxicity (OECD 402), acute inhalation toxicity (OECD 403), skin irritation (OECD 404), eye irritation (OECD 405), and skin sensitization (OECD 406) [14-18]. All studies were performed under controlled laboratory conditions, and test animals were maintained in accordance with applicable animal welfare regulations.

Toxicity endpoints, including LD_{50} and LC_{50} values, were determined using standard methods specified in the corresponding OECD guidelines. Observations of clinical signs and recovery behavior were recorded throughout the study period. Toxicity classifications were assigned according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS 2019) [19].

2.5. Data Analysis

Data analysis was primarily descriptive and focused on comparative evaluation of formulation-dependent differences. Physicochemical stability data were assessed by comparing changes in active ingredient content, suspensibility, and other quality parameters before and after storage. Toxicity data were interpreted according to regulatory classification criteria rather than statistical hypothesis testing, which is appropriate for acute toxicity assessment and formulation comparison studies.

3. Results and Discussion

3.1. Physicochemical Stability

The physicochemical stability of fluazinam formulations was evaluated under room temperature and accelerated storage conditions, and the results are summarized in Table 2. All tested formulations, including SC, WP, and WDG, maintained acceptable appearance and showed no visible signs of phase separation during storage. These observations indicate that the selected formulation components and processing methods were suitable for producing physically stable fluazinam products.

Table 2. Physicochemical stability of fluazinam formulations under different storage conditions

Formulation type	Storage condition	Appearance	Phase separation	Active ingredient content	Suspensibility	pH variation	Overall stability
SC	Room temperature	No change	None	Within acceptable range	Acceptable	Minor change	Stable
SC	54 °C, 14 days	No change	None	Within acceptable range	Acceptable	Minor change	Stable
SC	0 °C, 7 days	No change	None	Within acceptable range	Acceptable	Minor change	Stable
WP	Room temperature	No change	–	Within acceptable range	Acceptable	Minor change	Stable
WP	54 °C, 14 days	No change	–	Within acceptable range	Acceptable	Minor change	Stable
WDG	Room temperature	No change	–	Within acceptable range	Acceptable	Minor change	Stable
WDG	54 °C, 14 days	No change	–	Within acceptable range	Acceptable	Minor change	Stable

The active in ingredient content of all formulations remained within acceptable limits after storage, demonstrating satisfactory chemical stability of fluazinam in the tested systems. This result is particularly important for fluazinam, as chemical degradation during storage could lead to reduced efficacy and increased formation of undesirable by-products. The ability of all formulation types to maintain active ingredient content suggests that the selected formulation matrices provided adequate protection against chemical degradation under both ambient and accelerated conditions.

In terms of physical stability, suspensibility values of all formulations met the required criteria, indicating effective dispersion of fluazinam particles in water. However, formulation-dependent differences were observed in suspension behavior. The SC formulation exhibited consistently high suspensibility, which can be attributed to its finely controlled particle size distribution (D50 < 5 μm, D98 < 10 μm) and the presence of appropriate surfactants and rheology modifiers that help prevent particle aggregation and sedimentation. In contrast, WP and WDG formulations rely primarily on surface-active agents and disintegration processes to achieve dispersion, which may be more sensitive to formulation composition and particle size heterogeneity.

The SC formulation also demonstrated good stability under low-temperature storage conditions (0 °C for 7 days), with no evidence of irreversible aggregation or phase separation. This behavior highlights the importance of formulation design, particularly the selection of antifreeze agents and stabilizers, in maintaining suspension integrity under temperature fluctuations encountered during storage and transportation.

Overall, the stability results confirm that all three formulation types are technically feasible for fluazinam; however, the SC formulation shows advantages in terms of suspension stability and robustness against storage stress.

3.2. Acute Toxicity

The acute toxicity profiles of the tested formulations are summarized in Table 3. All formulations were classified as low acute toxicity according to GHS 2019 criteria, with oral and dermal LD_{50} values exceeding regulatory threshold levels. These results are consistent with previously reported toxicological data for fluazinam-based products.

Although the overall toxicity classifications were similar, formulation-dependent differences were observed in acute inhalation toxicity. The SC formulation exhibited a higher LC_{50} value compared with WP and WDG formulations, indicating lower inhalation toxicity under the test conditions. This difference may be attributed to the reduced potential for dust and fine particle generation in SC formulations, as the active ingredient is dispersed in a liquid medium rather than present as a dry solid.

Powder-based formulations such as WP are more prone to generating airborne particles during handling and mixing, which can increase inhalation exposure risk to operators. WDG formulations were developed to mitigate dust-related issues; however, aerosol formation may still occur during granule disintegration and dispersion, particularly if granule strength and disintegration behavior are not optimally balanced. The observed inhalation toxicity trends in this study are therefore consistent with known exposure characteristics of different formulation types.

These findings emphasize that, even when the same active ingredient is used, formulation design can significantly influence inhalation exposure and associated toxicological outcomes. Consequently, formulation-level evaluation is essential for a comprehensive assessment of pesticide safety.

Table 3. Acute toxicity classification of fluazinam formulations according to OECD guidelines and GHS 2019

Test type	Guideline	SC	WP	WDG
Acute oral toxicity (LD_{50}, mg/kg)	OECD 423	> 5000	> 5000	> 5000
Acute dermal toxicity (LD_{50}, mg/kg)	OECD 402	> 2000	> 2000	> 2000
Acute inhalation toxicity (LC_{50}, mg/L)	OECD 403	> 1.42	> 1.13	> 1.20
GHS acute toxicity category	GHS 2019	Category 4	Category 4	Category 4

Table 4. Skin and eye irritation and sensitization responses of fluazinam formulations

Test type	Guideline	SC	WP	WDG
Skin irritation	OECD 404	Non-irritant	Non-irritant	Non-irritant
Eye irritation	OECD 405	Category 2B	Category 2B	Category 2B
Skin sensitization	OECD 406	Negative	Negative	Negative
Recovery behavior	–	Complete recovery	Complete recovery	Complete recovery

3.3. Skin and Eye Irritation and Sensitization

The results of skin irritation, eye irritation, and skin sensitization studies are presented in Table 4. All formulations were classified as non-irritant to skin and showed no evidence of skin sensitization. Eye irritation responses were mild and reversible for all tested formulations, with no permanent damage observed during the recovery period.

Although all formulations fell within the same irritation classification, slight differences in response intensity and recovery behavior were noted. The SC formulation exhibited marginally lower conjunctival response scores and faster recovery compared with WP and WDG formulations. This observation may be related to differences in formulation composition and physical form, as liquid formulations generally allow more uniform dilution and distribution upon contact with ocular surfaces, potentially reducing localized irritation.

Powder-based formulations may lead to higher local concentrations upon contact prior to complete dispersion, which could contribute to transient irritation effects. While such differences were not sufficient to alter regulatory classification in this study, they highlight the role of formulation characteristics in influencing user experience and safety during accidental exposure.

3.4. Influence of Formulation Type on Exposure-Related Toxicological Behavior

Taken together, the stability and toxicity results demonstrate that formulation type plays an important role in shaping the practical safety profile of fluazinam-based products. While all tested formulations met basic regulatory requirements for stability and acute toxicity, the SC formulation consistently showed advantages in terms of suspension stability, reduced dust generation, and lower inhalation-related effects.

These findings are consistent with previous reports indicating that suspension concentrates often provide improved handling safety and reduced operator exposure compared with powder-based formulations. Importantly, this study confirms these general observations within the context of a single active ingredient formulated into multiple formulation types under standardized experimental conditions.

From an application and commercialization perspective, the results suggest that SC formulations may offer a favorable balance between stability, efficacy potential, and user safety for fluazinam. Nevertheless, WP and WDG formulations remain viable options, particularly where formulation simplicity, cost considerations, or specific application requirements are prioritized. Therefore, formulation selection should consider not only toxicological classification but also practical exposure characteristics and stability performance.

4. Conclusion

This study demonstrated that fluazinam can be successfully formulated into SC, WP, and WDG formulations at laboratory scale, all of which exhibited acceptable physicochemical stability and low acute toxicity in accordance with regulatory classification criteria. By evaluating formulation-dependent differences under standardized experimental conditions, the present work highlights the importance of formulation design in determining both stability performance and exposure-related toxicological behavior.

Although all tested formulations maintained active ingredient content and met suspensibility requirements after storage, distinct differences were observed in physical stability and inhalation-related toxicity. The SC formulation showed superior suspension stability and lower inhalation-related effects, which can be attributed to controlled particle size distribution and reduced dust generation compared with powder-based formulations. These findings confirm that formulation type can influence practical safety characteristics beyond the intrinsic toxicological properties of the active ingredient.

From a practical perspective, the results provide valuable information for the selection of suitable formulation strategies for fluazinam-based fungicides, particularly in the context of improving handling safety and reducing operator exposure. While WP and WDG formulations remain viable options under certain

conditions, the SC formulation appears to offer a favorable balance between stability, safety, and potential applicability for large-scale production.

Future studies may focus on further optimization of formulation composition, long-term storage stability, and evaluation of chronic or environmental toxicity to support comprehensive risk assessment and sustainable product development.

Declaration of Competing Interest

The authors declare no competing financial or personal interests.

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