

Conclusion

CONCLUSION

It could be concluded that:

- 1- Fungal species differ greatly in their resistance to gamma radiation.
- 2- *Curvularia lunata*, *Alternaria alternata* and *Fusarium oxysporum* were more radiation resistance than corresponding *C. tuberculata*, *A. tenuissima* and *F. semitectum*.
- 3- The relative high radiation resistant fungal species contained high protein content in addition to higher amino acids content particularly sulfur containing amino acids and double bond amino acids such as histidine.
- 4- The relative high radiation resistant fungi contained higher lipid content and fatty acid content specially unsaturated fatty acids.
- 5- The relative radiation resistant fungal species contained higher amount of nucleic acids (DNA and RNA).
- 6- Although the resistant species could resist the harmful effect of gamma irradiation but the results indicated that 12.5 kGy was quite sufficient to eliminate *C. lunata* completely from lupine seeds and *A. alternata* from chicken feed either after irradiation or during storage.
- 7- Irradiation dose of 10.0 kGy completely eliminated *F. oxysporum* contaminated chicken feeds and these feeds remained free from the spores of that fungi through out the storage period (3 months).

Summary

SUMMARY

There are many different methods used in inhibiting and controlling fungal growth in food and in agricultural commodities. Traditional methods using chemical compounds, heat and biological control have limited effect and many disadvantages as well. A modern, advanced and clean technology is now well established and known as "Food irradiation technology". This technology appeared to be effective and efficient in controlling fungi contaminating agricultural commodities. However, it was found that some fungal genera such as *Curvularia*, *Alternaria* and *Fusarium* are more resistant to ionizing radiation than others.

The aim of the present study was:

- 1- Isolation and identification of radiation-resistant fungi (*Curvularia* spp., *Alternaria* spp. and *Fusarium* spp.) from their natural products particularly foods, feeds, soil,etc.
- 2- Determine the "radiation decimal reduction dose" (D_{10} -value) of fungal spores to know the sensitivity or resistance of these molds to irradiation.
- 3- Examine the role of cellular composition (total proteins, amino acids, total lipids, fatty acids, DNA and RNA content) in the radiation-resistance.
- 4- Prevention of mold growth contaminating the food by using irradiation.

In this study, fifty three isolates of, *Curvularia* (C), *Alternaria* (A) and *Fusarium* (F) were isolated from different sources i.e. wheat (w), potato (p), tomato (t), mandarin (m), fenugreek (f), bread (b), orange (o), lupine (l), chicken feed (c), soil (s) and air (a). Five isolates were selected

from each genus according to the difference in the morphological characters and the source of food. The obtained results were summarized as follows:

- 1- The growth diameter of *Cl*₁ exposed to 10.0 kGy recorded 7.0 cm after 6 days of incubation while it reached 4.0 cm after 7 days of incubation in case of *Co*. Meanwhile, dose level 6.0, 8.0 and 9.0 kGy completely inhibited the growth of *Cl*₂, *Ca* and *Cs*, respectively.
- 2- The growth diameter of *Am* and *Af*, completely inhibited after exposing to 9.0 kGy, while *Ac* recorded 2.9 cm after 7 days of incubation at the same dose. Meanwhile 5.0 kGy completely inhibited the growth of *Ab* and *At*.
- 3- The growth diameter of *Fp*, *Ft*, *Fs* and *Fc* completely inhibited after exposing to 4.0, 7.0, 8.0 and 9.0 kGy, respectively after 7 days, while *Fw* completely inhibited at 6.0 kGy after 5 days.
- 4- Two isolates from each genus were chosen to represent the highest and lowest radiation resistance of the three genera for further studies. The relative resistant isolates identified as *Curvularia lunata* (*Cl*₁), *Alternaria alternata* (*Ac*) and *Fusarium oxysporum* (*Fc*), while the sensitive isolates identified as *C. tuberculata* (*Cl*₂), *A. tenuissima* (*At*) and *Fusarium semitectum* (*Fp*).
- 5- The radiation resistance of the six fungal species was studied as a function of biomass. The biomass of the six resistant and sensitive fungal species were decreased by increasing the dose level of radiation; dose level 8.0 kGy almost inhibited the growth of *C. tuberculata* and *F. semitectum* while it decreased the dry mass of *C. lunata*, *A. alternata*, *A. tenuissima* and *F. oxysporum* by 67.8, 47.8, 92.0 and 61.2%, respectively.

- 6- The radiation resistance of the six fungal species belonging to *Curvularia*, *Alternaria* and *Fusarium* was studied through determination of D₁₀-value. D₁₀-value (the radiation dose in kGy which kills 90 % of the initial count of the cells) of the six selected fungal species were studied. Treatment of fungal spores with radiation reduced their viable counts and this reduction was proportional with the irradiation dose. The D₁₀-values of *C. lunata*, *C. tuberculata*, *A. alternata*, *A. tenuissima*, *F. oxysporum* and *F. semitectum* in saline solution were found to be 1.92, 1.25, 1.47, 0.47, 1.31 and 0.70 kGy, respectively. Meanwhile, in lupine seeds, the D₁₀-values of *C. lunata* and *C. tuberculata* were 2.25 and 1.56 kGy respectively, and in chicken feed, the D₁₀-values of *A. alternata*, *A. tenuissima*, *F. oxysporum* and *F. semitectum* were 1.70, 1.30, 1.83 and 1.23 kGy respectively. It could be noticed that the D₁₀-values of these fungi under investigation were higher in substrates than saline solution.
- 7- The total protein content of the three relative resistant strains *C. lunata*, *A. alternata* and *F. oxysporum* were 76.88, 72.69 and 69.83%, respectively, while three relative sensitive species *C. tuberculata*, *A. tenuissima* and *F. semitectum* were less than the resistant once since they recorded 70.13, 64.06 and 46.88%, respectively.
- 8- The content of the total amino acids in *C. lunata*, *A. alternata* and *F. oxysporum* were 129.2, 114.4 and 49.2 mg/g, respectively, while in the relative sensitive strains: *C. tuberculata*, *A. tenuissima* and *F. semitectum* were 101.0, 74.0 and 63.2 mg/g, respectively. Meanwhile, the resistant strains: (*C. lunata*, *A. alternata* and *F. oxysporum*) contain higher content of sulfur containing amino acids (Cysteine, Methionine) or double bond amino acids

- (histidine) than the relative sensitive strains: *C. tuberculata*, *A. tenuissima* and *F. semitectum*.
- 9- The total lipids content of the highly relatively resistant strains: *C. lunata*, *A. alternata*, *F. oxysporum* were 16.26, 12.57 and 8.16%, respectively. Meanwhile, the total lipids in the relative sensitive strains: *C. tuberculata*, *A. tenuissima* and *F. semitectum* were less than the resistant once, since they recorded 6.99, 3.91 and 5.76 % respectively.
 - 10- The percentages of the total unsaturated fatty acids in the resistant strains: *C. lunata*, *A. alternata* and *F. oxysporum* were 73.48, 71.68 and 70.11 %, respectively, while in the relative sensitive strains: *C. tuberculata*, *A. tenuissima* and *F. semitectum* were less than the resistant once, since they recorded 69.11, 68.67 and 53.38 %, respectively.
 - 11- The total nucleic acids content of the resistant strains *C. lunata*, *A. alternata* and , *F. oxysporum* were 36.63, 35.13 and 33.41 mg/g, respectively, compared with 29.60, 28.17 and 28.46 mg/g for *C. tuberculata*, *A. tenuissima* and *F. semitectum*, respectively.
 - 12- Gamma irradiation with dose level 4.0 kGy decreased the total protein content, in the tested strains, especially in the sensitive ones since they recorded 9.45, 15.55 and 14.23 % for *C. tuberculata*, *A. tenuissima* and *F. semitectum*, respectively compared with 6.50, 8.52 and 9.42 % for *C. lunata*, *A. alternata* and , *F. oxysporum*, respectively in the relative resistant ones.
 - 13- Gamma irradiation with dose level 4.0 kGy decreased the content of the total amino acids to 109.6, 62.0, 62.6, 59.6, 36.6 and 55.6 mg/g in *C. lunata*, *C. tuberculata*, *A. alternata*, *A. tenuissima*, *F. oxysporum* and *F. semitectum*, respectively, especially, sulfur containing amino acids (cysteine, methionine) or double bond amino acids (histidine).

- 14- Gamma irradiation with dose level 4.0 kGy decreased the total nucleic acids content, in the tested strains, especially in the sensitive ones since they recorded 30.7, 46.75 and 34.29 % for *C. tuberculata*, *A. tenuissima* and *F. semitectum*, respectively compared with 20.28, 21.72 and 27.18% for *C. lunata*, *A. alternata* and , *F. oxysporum*, respectively in the relative resistant ones.
- 15- The values of RNA and DNA decreased by exposure the tested species to 4.0 kGy, but the percentage of decreasing were higher in DNA than RNA, also higher in the relative sensitive strains than the resistant strains. The percentage of decreasing recorded 32.8, 33.7 and 29.7 % in the resistant strains: *C. lunata*, *A. alternata* and *F. oxysporum*, respectively, compared with 40.6, 59.4 and 55.0 % in the sensitive strains *C. tuberculata*, *A. tenuissima* and *F. semitectum*.
- 16- The results of the storage experiment showed that the count of *C. lunata*, *A. alternata* and *F. oxysporum* (the relative resistant ones) artificially contaminated lupine seeds or chicken feeds increased after the first month either in the unirradiated or irradiated samples with doses (2.5-7.5 kGy). Thereafter the remain cells decreased gradually during the end of the storage periods (3 months). 10 kGy was sufficient to complete elimination of *F. oxysporum* contaminated chicken feeds, while 12.5 kGy completely freedom chicken feeds or lupine seeds from *A. alternata* and *C. lunata*, respectively.

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Arabic Summary



جامعة بنها
كلية العلوم
قسم النبات

مقاومة بعض الفطريات لأشعة جاما ودور المحتوى الخلوى فى مقاومة التشعيع

رسالة مقدمة من

رشا يحيى عبد الغفار محمد

بكالوريوس علوم . جامعة الزقازيق . فرع بنها (١٩٩٩)

للحصول على

درجة الماجستير فى الميكروبيولوجى

محت إشراف

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جامعة بنها
كلية العلوم
قسم النباتات

المحكمة

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عنوان الرسالة:

مقاومة بعض الفطريات لأشعة جاما ودور المحتوى
الخلوى فى مقاومة التشعيع

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الملخص العربي

يوجد العديد من الطرق لتثبيط النمو الفطري في الغذاء والمنتجات الزراعية ومنها استخدام المواد الكيميائية والحرارة والمكافحة البيولوجية حيث وجد أن لها تأثير محدود كما أن لها بعض المساوئ، أما الطريقة الحديثة وهي تكنولوجيا تشعيع الغذاء فهي طريقة متقدمة ونظيفة ومعتمدة من المنظمات الدولية المعنية ولكن وجد أن هناك بعض الفطريات لها قدرة نسبية على مقاومة الإشعاع مثل جنس الكيرفيولاريا، الألترناريا والفيوزاريوم، فهي تحتاج إلى جرعات مرتفعة للقضاء عليها.

ويهدف هذا البحث إلى عزل هذه الفطريات ودراسة مدى مقاومتها للإشعاع ودراسة محتواها الخلوي لمعرفة الدور الذي تلعبه هذه المحتويات في مقاومتها للإشعاع.

في هذه الدراسة تم الحصول على ٥٣ عزلة من جنس الكيرفيولاريا، الألترناريا والفيوزاريوم من مصادر مختلفة مثل القمح والبطاطس والطماطم واليوسفي والحلبة والخبز والبرتقال والتمرس وعلف الدواجن وأيضاً الهواء والتربة.

تم اختيار خمس عزلات من كل جنس طبقاً للفروق في شكلها الظاهري كذلك اختلاف المصدر المعزولة منه وكانت النتائج التي تم الحصول عليها كالتالي:

١- بالنسبة للعزلات المعزولة من جنس الكيرفيولاريا (C) كان قطر النمو في العزلة Cl_1 (المعزولة من التمرس) والمعرضة لجرعة إشعاعية ١٠ كيلو جراى (٧سم) بعد ستة أيام من التحضين، بينما وصل إلى ٤ سم بعد سبعة أيام من التحضين في حالة العزلة Co (المعزولة من البرتقال) في حين أن الجرعة ٦، ٨، ٩ كيلو جراى كانت قادرة على تثبيط نمو العزلات Cs, Ca, Cl_2 (المعزولة من التمرس والهواء والتربة) على التوالي.

٢- بالنسبة للعزلات المعزولة من جنس الألترناريا (A) تم تثبيط قطر النمو في العزلتين Am, Af (المعزولتين من الحلبة واليوسفي) بعد تعرضهم للجرعة ٩ كيلو جراى بينما كان قطر النمو في العزلة Ac (المعزولة من علف الدواجن) مازال ٢,٩ سم بعد سبعة أيام من التحضين عند نفس الجرعة. في حين أن الجرعة ٥ كيلو جراى كانت قادرة على تثبيط النمو في العزلتين Ab, At (المعزولتين من الطماطم والخبز).

٣- بالنسبة للعزلات المعزولة من جنس الفيوزاريوم (F) وجد أن الجرعات ٤، ٧، ٨، ٩ كيلو جرای كانت لها القدرة على تثبيط النمو في كل من العزلات الآتية Fc, Fs, Ft, (المعزولة من البطاطس والطماطم والتربة وعلف الدواجن) على التوالي بعد سبعة أيام من التحضين، بينما FW (المعزولة من القمح) يتوقف نموها تماماً بعد تعرضها إلى الجرعة ٦ كيلو جرای عند اليوم الخامس من التحضين .

٤- تم اختيار عزلتين من كل جنس ليمثلا العزلات المقاومة للإشعاع والحساسة له في الثلاثة أجناس المختبرة وعرفت العزلات المقاومة نسبياً للإشعاع كما يلي: كيرفيولاريا ليوناتا (Cl₁)، ألترناريا ألترناتا (Ac)، فيوزاريوم أوكسي سبورم (Fc). بينما عرفت العزلات الحساسة نسبياً للإشعاع كما يلي: كيرفيولاريا تيوبركيولاتا (Cl₂)، ألترناريا تينوسيما (At) وفيوزاريوم سيمي تيكتم (Fp).

٥- وجد أن الوزن الجاف للسلاسل المقاومة والحساسة للإشعاع يقل بزيادة الجرعة الإشعاعية حيث أن الجرعة ٨ كيلو جرای كانت قادرة على تثبيط النمو في فطر الكيرفيولاريا تيوبركيولاتا والفيوزاريوم سيمي تيكتم بينما نفس الجرعة أدت إلى انخفاض الوزن الجاف للكيرفيولاريا ليوناتا وألترناريا ألترناتا وألترناريا تينوسيما وفيوزاريوم أوكسي سبورم إلى: ٦٧,٨، ٤٧,٨، ٩٢,٥، ٦١,٢ % على التوالي.

٦- أوضحت النتائج أن الجرعات الإشعاعية اللازمة لقتل ٩٠ % من الجراثيم (قيمة الانخفاض العشري D₁₀) للسلاسل المختبرة: كيرفيولاريا ليوناتا، كيرفيولاريا تيوبركيولاتا، ألترناريا ألترناتا، ألترناريا تينوسيما، فيوزاريوم أوكسي سبورم وفيوزاريوم سيمي تيكتم كانت ١,٩٢، ١,٢٥، ١,٤٧، ١,٤٧، ٠,٤٧، ١,٣١، ٠,٧٠ كيلو جرای في المحلول الملحي على التوالي. في حين أن قيمة الانخفاض العشري في الكيرفيولاريا ليوناتا والكيرفيولاريا تيوبركيولاتا الملوثة لبذور الترمس كان ٢,٢٥، ١,٥٦ كيلو جرای على التوالي، أما في حالة ألترناريا ألترناتا، ألترناريا تينوسيما، فيوزاريوم أوكسي سبورم وفيوزاريوم سيمي تيكتم الملوثة لعلف الحيوان كانت قيمة الانخفاض العشري ١,٧٠، ١,٣٠، ١,٨٣، ١,٢٣ كيلو جرای على التوالي. مما يدل على اختلاف مقاومة هذه الأنواع لأشعة جاما وأن هذه المقاومة تزداد في الأوساط الغذائية عنها في المحلول الملحي.

٧- أوضحت النتائج أن كمية البروتين الكلي في السلاسل المقاومة للإشعاع وهي الكيرفيولاريا ليوناتا وألترناريا ألترناتا وفيوزاريوم أوكسي سبورم كانت ٦٧,٨٨،

٧٢,٦٩، ٦٩,٨٣ % على التوالى، أما فى حالة السلالات الحساسة نسبياً للإشعاع وهى كيرفيولاريا تيوبركيولاتا وألترناريا تينيوسىما وفىوزارىوم سيمى تىكتم فكانت قيمة البروتين الكلى لها أقل من السلالات المقاومة وقد سجلت النتائج الآتية ٧٠,١٣، ٦٤,٠٦، ٤٦,٨٨ % على التوالى.

٨- وجد أن المحتوى الكلى للأحماض الأمينية فى الفطريات المقاومة للإشعاع الكيرفيولاريا ليوناتا وألترناريا ألترناتا وفىوزارىوم اوكسى سبورم كان ١٢٩,٢، ١١٤,٤، ٤٩,٢ مليجرام / جرام على التوالى، بينما كان فى الفطريات الحساسة نسبياً للإشعاع كيرفيولاريا تيوبركيولاتا وألترناريا تينيوسىما وفىوزارىوم سيمى تىكتم ١٠١,٠، ٧٤,٠، ٦٣,٢ مليجرام/جرام على التوالى، وكانت كمية الأحماض الأمينية الكبريتية (سيسئين وميثيونين) وكذلك التى تحتوى على رابطة ثنائية (هستيدين) فى السلالات المقاومة نسبياً للإشعاع أكبر عند مقارنتها بالسلالات الحساسة نسبياً للإشعاع.

٩- سجلت الفطريات المقاومة للإشعاع نسبة دهون كلية أعلى مقارنة بالفطريات الحساسة نسبياً للإشعاع حيث كانت ١٦,٦، ١٢,٥٧، ٨,١٦ % على التوالى بينما كانت فى كيرفيولاريا تيوبركيولاتا وألترناريا تينيوسىما وفىوزارىوم سيمى تىكتم ٦,٩٩، ٣,٩١، ٥,٦٧ % على التوالى.

١٠- أظهرت النتائج أن نسبة الأحماض الدهنية غير المشبعة فى السلالات المقاومة للإشعاع (الكيرفيولاريا ليوناتا وألترناريا ألترناتا وفىوزارىوم اوكسى سبورم) كانت ٧٣,٤٨، ٧١,٦٨، ٧٠,١١ % على التوالى وقد كانت أعلى فى قيمتها من السلالات الحساسة نسبياً للإشعاع كيرفيولاريا تيوبركيولاتا وألترناريا تينيوسىما وفىوزارىوم سيمى تىكتم حيث كانت ٦٩,١١، ٦٨,٦٧، ٥٣,٣٨ % على التوالى.

١١- الفطريات المقاومة للإشعاع: الكيرفيولاريا ليوناتا وألترناريا ألترناتا وفىوزارىوم اوكسى سبورم وجد أنها تحتوى على نسب أعلى فى الأحماض النووية مقارنة بالفطريات الحساسة للإشعاع فكانت ٣٦,٦٣، ٣٥,١٣، ٣٣,٤١ مليجرام / جرام على التوالى، بينما كانت فى كيرفيولاريا تيوبركيولاتا وألترناريا تينيوسىما وفىوزارىوم سيمى تىكتم ٦٩,٦٠، ٢٨,١٧، ٢٨,٤٦ مليجرام / جرام على التوالى.

١٢- من النتائج السابقة يتضح أن السلالات المقاومة للإشعاع الكيرفيولاريا ليوناتا وألترناريا ألترناتا وفيوزاريوم أوكسى سبورم تحتوى على نسب أعلى من البروتين والدهون الأحماض الدهنية غير المشبعة والأحماض النووية وكانت قيمة الأحماض الأمينية الكبريتية (سيستين ، ميثيونين) وتلك التى تحتوى على رابطة ثنائية (هستيدين) أكبر فى السلالات المقاومة للإشعاع.

١٣- عند دراسة تأثير أشعة جاما عند الجرعة ٤,٠ كيلوجراى تناقصت كمية البروتين الكلى فى السلالات المختبرة وخاصة فى السلالات الحساسة نسبياً للإشعاع: (كيرفيولاريا تيوبركيولاتا وألترناريا تينوسيمما وفيوزاريوم سيمى نيكتم) حيث تناقصت بنسبة ٩,٤٥ ، ١٥,٥٥ ، ١٤,٢٣ % على التوالى، بينما فى السلالات المقاومة نسبياً للإشعاع: (الكيرفيولاريا ليوناتا وألترناريا ألترناتا وفيوزاريوم أوكسى سبورم) كان مقدار الإنخفاض ٦,٥٠ ، ٨,٥٢ ، ٩,٤٢ % على التوالى.

١٤- الجرعة ٤,٠ كيلوجراى أدت إلى خفض المحتوى الكلى للأحماض الأمينية فى فطريات كيرفيولاريا ليوناتا، كيرفيولاريا تيوبركيولاتا، ألترناريا ألترناتا، ألترناريا تينوسيمما، فيوزاريوم أوكسى سبورم وفيوزاريوم سيمى نيكتم إلى ١٠٩,٦ ، ٦٢,٠ ، ٦٢,٦ ، ٥٩,٦ ، ٣٦,٦ ، ٥٥,٦ على التوالى، وخاصة الأحماض الأمينية الكبريتية (سيستين وميثيونين) وتلك التى تحتوى على الرابطة الثنائية (هستيدين).

١٥- الجرعة ٤,٠ كيلوجراى خفضت نسبة الأحماض النووية الكلية للسلالات المختبرة خاصة فى السلالات الحساسة نسبياً للإشعاع: (كيرفيولاريا تيوبركيولاتا وألترناريا تينوسيمما وفيوزاريوم سيمى نيكتم) حيث سجلت ٣٠,٧ ، ٤٦,٧٥ ، ٣٤,٢٩ % على التوالى، بينما فى السلالات المقاومة نسبياً للإشعاع: (الكيرفيولاريا ليوناتا وألترناريا ألترناتا وفيوزاريوم أوكسى سبورم) كانت ٢٠,٨ ، ٢١,٧٢ ، ٢٧,١٨ % على التوالى.

١٦- انخفضت قيمتى كل من DNA, RNA بعد تعرض السلالات المختبرة إلى ٤,٠ كيلوجراى وكانت نسبة النقص فى DNA أعلى من مثيلتها فى RNA وكذلك كانت أعلى أيضاً فى السلالات الحساسة نسبياً للإشعاع عنها فى السلالات المقاومة للإشعاع حيث سجلت نسبة النقص فى DNA فى حالة الفطريات المقاومة للإشعاع كيرفيولاريا ليوناتا، ألترناريا ألترناتا ، فيوزاريوم أوكسى سبورم ٣٢,٨ ، ٣٣,٧ ، ٢٩,٧ % على التوالى، بينما كانت فى الفطريات الحساسة للإشعاع

كيرفيولاريا تيوبركيولاتا، ألترناريا تينيوسىما، وفيوزاريوم سىمى تىكتم كالآتى:
٤٠,٦ ، ٥٩,٤ ، ٥٥,٠ على التوالى.

١٧- أجريت تجربة تطبيقية لتحديد الجرعات الإشعاعية اللازمة لوقف نمو جراثيم الفطر كيرفيولاريا ليوناتا الملقحة فى بذور الترمس (وهو الأكثر مقاومة للإشعاع) كذلك فطريات ألتراناريا ألترناتا، فيوزاريوم اوكسى سبورم الملقحة فى علف الدواجن لمدة ثلاثة شهور وقد أوضحت النتائج أن العدد الكلى لجراثيم فطر الكيرفيولاريا ليوناتا وألترناريا ألترناتا وفيوزاريوم اوكسى سبورم (السلالات المقاومة نسبياً للإشعاع) يزداد بعد مرور شهر من التخزين سواء فى العينات غير المشععة أو المشععة بالجرعات (٢,٥-٧,٥ كيلو جراى) ثم يتناقص العدد الكلى أثناء فترة التخزين وكانت الجرعة ١٠,٠ كيلو جراى كافية لوقف نمو فطر فيوزاريوم اوكسى سبورم فى العلف سواء بعد التشعيع أو أثناء فترة التخزين بينما كانت الجرعة ١٢,٥ كيلوجراى كافية لخلو علف الدواجن من جراثيم فطر ألترناريا ألترناتا وبذور الترمس من فطر كيرفيولاريا ليوناتا أثناء فترة التخزين.