SECTIONS

INSECT INFESTATION OF DRUGS.*[1]

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INTRODUCTION.

Records of insect infestation in drugs during the last sixty years have been numerous. Unfortunately, however, the majority of these records remain scattered throughout the literature, and represent observations on the material at hand without any attempt to systematize, corroborate or extend the data previously accumulated. Some workers such as Sayre (4), Tschirch (5) and Stuhr (1) have made compilations dealing with many of the drugs liable to insect attack, a simplified (for pharmaceutical purposes) entomological discussion of the pests, and methods of control. Many of the textbooks on Pharmacognosy have treated this subject similarly, but all have, for the most part, omitted the purely pharmacognostical aspects of the topic. Information as to the chemical and botanical alterations produced by infestation, the identification of insect fragments in drugs, etc., is disconcertingly meager.

The importance of the subject is adequately recognized in the General Notices of the U. S. P. XI (6) wherein it is stated that vegetable drugs are to be as free as practicable from insects and other animal life, animal material or animal excreta. The same reference states that the following drugs are particularly liable to the attack of insects: aconite, belladonna root, cantharides, capsicum, caraway, cardamon, ergot, ginger, glycyrrhiza, linseed, myristica, rhubarb and sarsaparilla.

In the same section of the Pharmacopœia there are directions for the preservation of drugs against insect attack, but nowhere is there an analytical procedure for determining the presence of insects or their fragments in drugs, or a general paragraph describing the distinguishing features of a powdered insect infested drug. This statement also applies to the standard unofficial books often consulted by the pharmacognocist.

The purpose of this paper is to review the literature available on the pharmacognostical aspects of infestation, and to present experimental data on the rôle of *Tribolium* beetles as drug pests. It is realized that the present report does not follow through several of the problems which have arisen in connection with the project, but it is hoped that in the future, research will be done on some of the points mentioned; inclusion in pharmacognosy texts of the results accumulating therefrom should be very enlightening and of practical value.

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INFESTING INSECTS AND DRUGS ATTACKED.

From a bibliographical research it has been possible to compile a list of forty-seven infesting insects and eighty-two drugs which they attack. Seven miscellaneous items of pharmaceutical interest which are subject to attack, and eleven drugs resistant to infestation, have likewise been compiled. Owing to limitations of space, these cannot be included here. Interested readers may communicate with us for further details.

CONSTITUENTS AND STRUCTURES REMOVED FROM DRUGS BY INSECTS.

Stuhr in a recent investigation (1) says that in general those drugs rich in starch, inulin and sugars are most liable to insect attack. His opinion, especially as concerns starch, seems to be a well established fact, although one finds few experiments to substantiate it. On the other hand, an opinion to the contrary, on the basis of experimentation, has been expressed by Greenish and Braithwaite (2). These two workers cite the following results:

Examination of the powder collected from the bottom of a jar containing ginger infested with *Sitodrepa panicea* "shows that the powder consists almost entirely of small pellets of the excrement of the larvæ. . . . We were surprised to find that it consisted almost wholly of starch, some few grains of which showed evidence of partial digestion. While, therefore, the larva undoubtedly ingests considerable quantities of starch, only a small proportion of this would appear to be digested. Similarly, larvæ living on dandelion root excrete abundant fragments of tissue in which inulin is still to be found. In the case of coriander fruit, the endosperm upon which the larva feeds is free from starch, but contains fixed oil and abundant aleurone grains, in each of which a rosette of calcium oxalate is to be seen embedded in ground substance; the quantity of excrement is small and consists of portions of various tissues together with some oil globules and numerous calcium oxalate rosettes now devoid of nitrogenous substances such as the remains of protoplasm, etc."

The presence of the lactiferous vessels of dandelion root in the excrement lead the same workers to doubt the correctness of the statement that the larvæ avoid those portions of the drug containing the active constituents. They further state that in aconite and belladonna roots, and doubtless others in which the alkaloids are stored in the parenchymatous tissue within the cork and in that near the bast ring, it is quite probable that the tissues are ingested by the larva when boring into the root.

Greenish and Braithwaite's conclusions are subject to some criticism. Inasmuch as ginger contains about 45% starch, it is possible that large quantities of the starch could be digested by the beetles and that it would still preponderate in the excreta. Carefully controlled quantitative experiments would seem necessary to justify the conclusions that a considerable amount of the carbohydrates of ginger are not utilized by insects. The same comment may apply to taraxacum which contains about 25% inulin. In the case of coriander it is quite probable that the beetle utilized the nitrogenized aleurone grains and some of the oily or fatty substances.

Notwithstanding these experiments, the results of our research appear to indicate that fair amounts of carbohydrates (especially starch) are consumed by the insects. However, it must not be assumed that carbohydrates alone are an adequate diet; they probably are supplemented by either proteins and/or fats if the beetle is to enjoy a normal life cycle.

Maier and Ballard (7) undertook a chemical and microscopical examination of wormy rhubarb to determine its anthraquinone content. The investigation was initiated after a discussion with a dealer who said that wormy belladonna roots and aconite contain relatively more alkaloid than the sound drug, because the starch was consumed by the insect. They observed that the starch content decreased and the fibrovascular tissues, parenchyma and calcium oxalate increased as the infestation progressed. The anthraquinone content increased relatively to a certain limit beyond which there was a diminishing, probably due to decomposition. The laxative properties were never entirely lost even in very old and wormy specimens.

The diminution of starch concomitant with infestation was confirmed by Denston (3) who examined licorice stolons infested by a species of *Lyctus*. Borings were present mainly in the xylem and pith. Powders from the borings were examined microscopically and showed the presence of circular or oval masses about 80μ in diameter and smaller fragments not exceeding 15μ. On disintegrating the masses with chloral hydrate, fragments of cellulosic parenchyma, lignified fibers and thickenings of the vessel walls were evident; the latter greatly predominated. Fiber fragments were relatively few and there was little calcium oxalate. The proportion of starch in the powder was considerably less than in normal licorice. The most characteristic feature was the fragments of reticulate and pitted walls of vessels.

Denston also reports the microscopical examination of a coarse, somewhat granular, dark green powder formed in stramonium by *Ptinus tectus*. The material was almost entirely in narrow rectangular pieces 300–600μ long and 50–120μ wide. Pieces had been cut from the leaves and flowers in irregular directions, but the midribs and petioles did not appear to have been attacked. Thus the powder showed all the elements of stramonium leaf and flower except the larger vessels such as are present in the midrib and petiole.

PHYSICAL CHANGES ASSOCIATED WITH INFESTATION.

The physical changes identifiable in whole and powdered drugs include web formation, borings and tunnels and gnawings. Such alterations usually result in the formation of powder.

ANALYTICAL PROCEDURE FOR IDENTIFICATION OF INSECT FRAGMENTS IN DRUGS.

The only analytical procedure that this author has found in the literature for the identification of insect fragments in drugs is that of Greenish and Braithwaite (2). They prepared powders from infested crude drug stock and employed the following method:

Defat 5 Gm. of powder with ether in a Soxhlet; dry the defatted powder and boil with 100 cc. of 5% HCl for five minutes in a tared flask; add about 150 cc. water, allow the powder to settle and wash once by decantation. For every 35 Gm. of water and powder in the flask, add 6 cc. conc. H_2SO_4, cool, and then add in small portions and cooling again if there is any considerable rise in temperature, 10 cc. of a 1 in 1 aqueous solution of chromic acid. Allow the mixture to stand with occasional agitation for 36 hours or longer. Separate the solid particles by centrifugation, wash then with water, alcohol and ether successively, dry, remove from tube and mount in xylol balsam.

Variations in the method may be necessary with different powders; thus, if the drug contains little material that is ether soluble the treatment with this solvent may be omitted, and similarly that with HCl. The residue in the centrifuge tube may be examined at once under the microscope if a permanent record is not desired. Eighteen hours often suffices, but a better result is obtained by allowing the oxidizing mixture to act 36–48 hours after which time the residue will consist of little else than sand and beetles. The particles of beetles are readily detected by their conspicuous color, and most of them will exhibit either hairs or scars of hairs.

This method was tested quantitatively by mixing 5 Gm. of powdered rhubarb with 0.00001 Gm. of *Sitodrepa* (contained in a milk sugar trituration). Since the authors had previously studied the covering parts of the *Sitodrepa panicea* body microscopically, they could accurately identify the several fragments of beetle contained in the residue. It would appear that one mature beetle in every Gm. of powder is indicative of a highly infested condition.

The authors also include a table of results for ten different powdered drugs and spices tested by this method.

The principal shortcoming of this method is that it does not attempt to detect insect eggs or the softer parts of the immature forms of the insect. Detection of the eggs may be of considerable practical importance, when an insect infested drug has been so reconditioned that all of the larger forms are removed.

Tribolium INFESTATION IN DRUGS.

Since *Tribolium* beetles have not heretofore received much consideration as drug pests, one may wonder why we have experimented with these beetles. Our investigation seems justified in view of the following: (1) The losses caused by insects injurious to stored grain and milled products in the United States represent about $250,000,000 annually. According to one report (14) 84.65% of all species of insects removed from mill streams of flour mills during 1934–1935 were *Tribolium*. (2) We have quite frequently found dead *Tribolium* beetles in our own drug stock, although the only drug with which live insects seem to have been definitely associated was linseed. (3) There are records of the occurrence and depredations of *Tribolium* from practically every civilized country in the world. Its ubiquity alone may make it a potential drug infester. (4) According to previous reports (8, 9) *Tribolium confusum* has been bred in orris root and in cayenne pepper with some difficulty; it feeds on nutmeg, mustard, ginger and cinnamon, and may utilize elm bark as a source of food.

Probably the most serious insect drug pest is *Sitodrepa panicea* (the drug store beetle); its omnipresence and destructiveness in drug fields closely parallels that of *Tribolium* (the flour beetle) in grains and stored products.

EXPERIMENTAL DATA.

Procedure.—The same method of handling, counting, etc., was employed throughout our experiments, the only variable factor being the type of receptacle in which the drugs were contained. In earlier tests we used 1 by 4 inch vials for the powders and four-ounce wide-mouthed jars for the whole drugs; each container was loosely stoppered with cotton. Because of the difficulty in finding and counting the beetles, we next tried 100 cc. unstoppered beakers for holding the powders; these permitted a more facile handling of the insects than the vials, but still did not seem completely satisfactory. We finally resorted to using 10 cm. petri dishes, and found them very suitable. In many cases the ten beetles used in each experiment could be observed in the petri dishes without disturbing the substrate. No specific experiments were run to determine whether the difference in air and moisture exchange in a closed petri dish as compared with that in a cotton-stoppered vial or an open beaker would affect the longevity of the beetles. However, we consider this factor negligible inasmuch as we have kept *Tribolium* adults alive for more than eight months in closed petri dishes.

The beetles used in the experiments were cultured in either wheat or barley flour kept in a dark place at room temperature (21–23° C.). Whenever insects were required for experimentation, teaspoonfuls of the infested flour were transferred to a 4XX silk bolting cloth, most of the flour was removed by very gentle sifting, and then the beetles were dislodged into a petri dish by means of a small camel's hair brush. It was usually necessary to transfer the insects from this dish to a second in order to remove all the adherent flour and extraneous material. There was no attempt to select insects of a definite age or sex; those used were apparently healthy, normal, active adults.

The drugs employed were of U. S. P. quality and showed no signs of insect infestation as revealed by a thorough macroscopic examination, although beetles other than *Tribolium* developed in some of the orris and ginger at a later date (see Table V). Approximately ten Gm. of all drugs, whole and powdered, were used.

For each trial ten beetles were placed in a petri dish with ten Gm. of the drug. As indicated in the tables of results, some dishes were kept at room temperature (21–23° C.), and others were placed in a thermostatically controlled incubator at 26–28° C. Mortality examinations were conducted by making an actual count of the number alive and dead, taking care not to disturb the individuals unnecessarily. (Chapman (10) has found that frequent handling increases the death rate of the beetles.) The mortality figures listed do not represent the maximum time that a certain number of beetles lived, but rather the number of beetles dead at the time observed.

RESULTS.

TABLE I.—10 GM. MATERIAL—10 BEETLES—DARK PLACE—IN UNSTOPPERED BEAKERS—POWDERED DRUGS AT 26–28° C.

Substance.	Mortality in Days. 4.	6.	11.	22.	33.	60.
R. rhaponticum—SBP	3	8	10	..	..	..
R. rhaponticum—CUCP	1	4	10	..	..	..
Krameria	2	10	..	..	..	..
Geranium	10	..	..	..	..	..
R. officinale—SBP No. 3344	3	8	10	..	..	..
R. officinale—CUCP	3	4	10	..	..	..
R. officinale—No. 129	6	9	10	..	..	..

TABLE I.—10 GM. MATERIAL—10 BEETLES—DARK PLACE—IN UNSTOPPERED BEAKERS—POWDERED DRUGS AT 26–28° C. (*Continued from page 486.*)

Substance.	Mortality in Days. 4.	6.	11.	22.	33.	60.
R. officinale—CWB No. 10307	4	7	9	10	..	..
R. officinale—CWB No. 10307	..	..	8	10	..	..
R. officinale—SBP No. 51972	3	8	10	..	..	..
Rubus	1	5	10	..	..	..
Tannic acid 10%—corn starch	..	..	..	9	10	..
Tannic acid 15%—corn starch	..	..	..	9	10	..
Tannic acid 20%—corn starch	..	3	6	10	..	..
Aloin 1.5%—corn starch	0	0	0	1	1	1
Aloin 3.0%—corn starch	0	0	0	3	3	3
Aloin 4.5%—corn starch	0	0	1	3	3	9
Calcium oxalate 3%—corn starch	0	1	2	6	7	8
Calcium oxalate 5%—corn starch	0	0	0	3	6	9
Calcium oxalate 7%—corn starch	0	0	1	2	5	8
Tannic acid 15%, calcium oxalate 5%, corn starch 80%	0	1	6	10	..	..
Tannic acid 15%, calcium oxalate 7%, corn starch 75%, aloin 3%	0	4	10	..	..	..
Corn starch (control)	..	..	..	2	2	3

TABLE II.—SAME CONDITIONS AS TABLE I, BUT AT 21–23° C.

Substance.	Mortality in Days. 4.	6.	11.	22.	33.
R. rhaponticum	..	3	7	10	..
R. officinale—SBP No. 3344	..	1	5	10	..
R. officinale—SBP No. 51972	..	1	5	10	..
Krameria	..	..	2	8	10
Geranium	2	..	10	..	..
Rubus	..	..	9	10	..

TABLE III—SAME CONDITIONS AS TABLE II.

Substance.	Mortality in Days. 7.	15.	35.	37.	45.	59.
R. officinale—No. 129	0	0	..	10	..	..
R. officinale—No. 129	..	..	10	..	..	..
Aloin 1.5%—corn starch	0	0	..	..	3	3
Aloin 3.0%—corn starch	0	0	..	..	2	2
Aloin 4.5%—corn starch	0	0	..	..	2	2
Tannic acid 5.0%—corn starch	0	0	..	..	2	2
Tannic acid 10.0%—corn starch	0	0	..	..	2	*
Tannic acid 15.0%—corn starch	0	0	..	..	8	*
Corn starch (control)	0	0	..	..	1	2

* Dishes accidentally destroyed.

TABLE IV.—10 GM. MATERIAL—10 BEETLES—IN COTTON-STOPPERED JARS AND VIALS—26–28° C.

	Whole Drugs. Mortality in Days.								Powdered Drugs. Mortality in Days.							
Substance.	14.	21.	26.	31.	45.	53.	67.	83.	12.	21.	33.	38.	52.	60.	74.	90.
Aconite	10	..	..	..	..	..	..	..	10	..	..	..	..	..	..	..
Althea Root	5	..	..	10	..	..	..	..	6	8	8	9	9	..	..	9
Belladonna Root	5	..	9	10	..	..	..	..	10	..	..	..	..	..	..	..
Cantharides	..	4	10	..	..	..	..	..	9	..	..	10	..	..	..	..
Capsicum	..	10	..	..	..	..	..	..	9	..	..	10	..	..	..	..
Caraway	..	10	..	..	..	..	..	..	6	8	10	..	..	..	..	..

TABLE IV.—10 GM. MATERIAL—10 BEETLES—IN COTTON-STOPPERED JARS AND VIALS—26–28° C.

(*Continued from page 487.*)

Substance.	Whole Drugs. Mortality in Days. 14.	21.	26.	31.	45.	53.	67.	83.	Powdered Drugs. Mortality in Days. 12.	21.	33.	38.	52.	60.	74.	90.
Cardamon (whole)	..	10	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cardamon (decorticated)	..	10	..	..	..	..	..	..	1	3	4	5	6	7	7	9
Ergot	..	6	6*	..	6*	6[2]	[1]	[1]	3	4	4	4*	4*	4	[1]	[1]
Ginger (Jamaica)	6	7	9	..	9	10	..	..	..	..	..	..	..	..	..	..
Linseed	..	6	8	..	9*	..	..	..	..	..	..	..	..	..	..	..
Myristica (limed)	10	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Myristica (washed)	..	9	10	..	..	..	..	..	..	..	..	..	..	..	..	..
Rhubarb	10	..	..	..	..	..	..	..	10	..	..	..	..	..	..	..
Sarsaparilla (Mex.)	10	..	..	..	..	..	..	..	10	..	..	..	..	..	..	..
Sarsaparilla (Hond.)	10	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Glycyrrhiza (Span.)	10	..	..	..	..	..	..	..	10	..	..	..	..	..	..	..
Glycyrrhiza (Russ., whole)	10	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Glycyrrhiza (Russ., cut)	..	8	10	..	..	..	..	..	10	..	..	..	..	..	..	..
Althea Leaf	10	..	..	..	..	..	..	..	10	..	..	..	..	..	..	..
Corn starch (control)	..	..	..	..	..	..	..	..	1	1	..	..	2	2	4	4

* Live larvæ present

[1] New adult present

[2] One pupa present

TABLE V.—10 GM. MATERIAL—10 BEETLES—DARK PLACE—IN PETRI DISHES—26–28° C.

Substance.	Whole Drugs. Mortality in Days. 11.	22.	36.	52.	Powdered Drugs. Mortality in Days. 11.	22.	36.	52.
Pillsbury 4X Flour (control)	..	..	..	..	..	1[1]	1	1
Pillsbury 4X Flour (control)	..	..	..	..	..	1[1]	1	2
Corn starch (control)	..	..	..	..	..	0	1	3
Corn starch (control)	..	..	..	..	..	1	2	3
Orris Root	5[3]	6	8[3]	8[3]	3	8	10	..
Orris Root	1	6	8[4]	10	4	7	9	9
Ginger (Jamaica)	7	7	9	10	4	7	10	..
Ginger (Jamaica)	3	6	7	7[5]	7	10	..	..
Capsicum	5	10	..	..	10	..	..	..
Capsicum	8	10	..	..	10	..	..	..
Ulmus	1	10	..	..	9	10	..	..
Ulmus	6	10	..	..	10	..	..	..
Myristica	..	..	..	..	6	10	..	..
Myristica	..	..	..	..	6	10	..	..
Mustard (black)	6	10	..	..	10	..	..	..
Mustard (black)	6	10	..	..	8	10	..	..
Cinnamon	10	..	..	..	10	..	..	..
Cinnamon	9	10	..	..	9	10	..	..
Linseed	1	2[2]	3[2]	3[1]	2	4[2]	6	6[1]
Linseed	1	3	5	5	1	1[2]	2	*

* New adult present.

[1] Heavily infested with larvæ.

[2] Live larvæ present.

[3] One live larva present. Matured at 36 days; adult not *Tribolium.*

[4] One live beetle (not *Tribolium*) present.

[5] Two live beetles (not *Tribolium*) present.

DISCUSSION OF RESULTS AND CONCLUSIONS.

Before proceeding with a general discussion of the results, it should be explained that our inference as to whether or not a drug was favorable to *Tribolium* nutrition, was based on two facts—the results in the control, and a statement by Good (9) who has observed that the average life of the adult beetles is more than one year, and that adults may resist starvation from fourteen to fifty-one days.

The experiments in Tables I, II and III were undertaken in an attempt to determine what constituents of rhubarb were toxic to *Tribolium* Before this investigation was initiated it was expected that rhubarb would be susceptible to attack by *Tribolium.* After testing several different samples and finding them toxic, we made chemical mixtures using the proportions of the more important substances present in rhubarb as a basis. From the results in these tables, it may be inferred that tannic acid either in artificially or naturally occurring mixtures is unfavorable to *Tribolium;* its toxicity increases directly as the temperature. Aloin is practically innocuous at either 21–23° C. or 26–28° C., but seems to increase the toxicity of tannic acid and calcium oxalate in admixture with the two. Calcium oxalate seems to have a decidedly detrimental effect on *Tribolium.*

R. rhaponticum gives almost identical mortality results with *R. officinale.* Probably the toxicity of neither is influenced by alœemodin, emodin, rhein or rhaponticin, since the latter only is present in *R. rhaponticum* while the other three are found in *R. officinale* (11).

The tannic acid content of *R. officinale, R. rhaponticum,* geranium, krameria and rubus varies from 8–28%. All of these drugs are resistant to attack by *Tribolium,* and with the exception of the first two, all are resistant to insect infestation generally. While tannic acid may be a contributory factor, present experiments are insufficient to permit definite conclusions, and previous literature would seem to negate this idea. For example, Williams (12) states that Felt defines galls as "vegetable excrescences resulting from insect activity and usually sheltering immature states of the producers." He also notes that beetles feeding on plants frequently produce galls which they eat. Since galls generally have a high tannic acid content (13), it would seem unlikely that this substance is unfavorable to them.

Future breeding experiments should employ the marc of the drug as a diluent for an artificial drug mixture, instead of starch; while adult *Tribolium* beetles feed upon and enjoy a good span of life in corn starch, this writer has never found that they lay eggs or multiply in it in the time noted.

Table IV represents experiments on the drugs, in crude and powdered form, recorded in the general notices of the U. S. P. XI as being liable to insect attack. With the exception of ergot and linseed (see Tables IV and V), none of these drugs is subject to attack by *Tribolium.* Crude ginger and powdered althea root may be utilized (because of their starch?) for relatively long periods, but the beetles will not pass through a complete life cycle on either of these drugs.

The constituents of this group of drugs are so varied chemically that it is impossible at this time to theorize on their relation to drug susceptibility or resistance.

Table V was conducted for the purpose of verifying previous observations. (See section on *Tribolium* Infestation in Drugs.) Contrary to such observations it was found that *Tribolium* will not live in whole or powdered capsicum, elm bark, myristica, mustard and cinnamon. Orris root, whole and powdered, seems to sustain life for a short time; linseed, whole and ground, permits feeding and breeding. Powdered ginger killed all the beetles promptly, but the results on whole ginger were somewhat vitiated by the presence of other insects (see Table V). Whereas our experiments would seem to indicate that the beetles starve to death on elm bark, Good (9) found that *Tribolium* could utilize this substance as an auxiliary food.

Perhaps the failure to breed *Tribolium* on capsicum was due to the fact that only ten beetles were employed in a single test. However, the same results obtained in four trials. Good (9) has bred this beetle in capsicum with some difficulty.

Of all the drugs included in the experiments, ergot and linseed were the only sufficiently favorable substrates for reproduction of the beetles.

SUMMARY.

1. An extensive list of insects attacking drugs and the specific drugs which they attack has been compiled from various sources.

2. The literature on the physical changes produced in drugs during infestation, and the chemical constituents and structures removed from drugs by insects, is presented. In general it would seem that carbohydrate containing drugs are quite susceptible to insect attack, but if other beetles, *e. g.*, *Sitodrepa panicea* may be judged by *Tribolium*, carbohydrates are not the only substances required. Protein and/or fats are also necessary.

3. *Tribolium* is not a serious drug pest; of the thirteen drugs listed in U. S. P. XI as being especially liable to insect attack, only ergot and linseed permitted this beetle to reproduce. Orris, ginger, althea root and decorticated cardamom sustained life for varying lengths of time.

4. Contrary to previous reports *Tribolium* could not be cultured in capsicum, nutmeg, cinnamon or mustard, and does not appear to utilize elm bark as an auxiliary food.

5. Tannic acid and calcium oxalate individually are unfavorable to *Tribolium*, while aloin has little effect.

6. It is recommended that further work be done on the pharmacognostical aspects of insect infested drugs with a view to furnishing more complete information on the chemical and botanical alterations produced in such drugs. This may ultimately influence official descriptions and standards. It is also suggested that an analytical method for the identification of insect fragments and eggs be perfected and included in standard pharmacognosy books. One such method previously devised is reviewed here.

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