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EFFECTS OF CERTAIN ALKALOIDS, GLUCOSIDES AND OTHER SUBSTANCES UPON THE INFECTIVITY OF THE MOSAIC TOBACCO JUICE

BY

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煙草のモザイク病の病原体に及ぼすアルカロイド、
グルコサイド其他の物質の影響

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Introduction

This investigation was undertaken in the hope of throwing some light on the nature of the virus of the mosaic disease of tobacco. In spite of an extensive literature concerning the virus diseases of plants and of the rapidly increasing number of pathologists whose attention has been attracted to study on this subject, comparatively little work has been done as regards the effects of toxic substances on the viruses, except ALLARD's work with the virus of the tobacco mosaic disease. It may be partly attributed to the difficulties which are encountered when we attempt to study the properties of the mosaic viruses. For the present, the mosaic viruses can not be isolated in a pure state and accordingly their natures must be studied in association with the juice of the host plant which is complex in nature. Under such circumstance, it is difficult to know what influence it may exert on the virus when treated with a certain chemical agent. It can be considered that some chemicals may act upon the ingredients of the plant fluid which contains the virus and a substance or substances thus produced may exert more or less influence on the behavior of

the virus, while the other chemicals may be adsorbed more easily by the colloidal particles in the fluid to reduce their effects on the virus. Thus the results of investigations along this line are apt to be considered rather uncertain and less significant. Nevertheless it can not be denied that such work will lead to the discoveries which are very suggestive in respect to the nature of the virus, to its classification, and to the development of more satisfactory experimental methods. The effects of ordinary toxic substances on the virus of the tobacco mosaic disease have been studied by RACIBORSKI (17), KONING (12), HEINTZEL (11), CLINTON (6), CHAPMAN (5), and ALLARD (1, 2, 3, 4) but none of these investigators has mentioned as regards the effect of alkaloids, glucosides, and etherial oils on the virus, with the exception of ALLARD (4) who stated that quinine bisulphate in 4 % strength did not affect the infectivity of the virus after 19 days' treatment while saponin in concentrations of 1, 2, 5, 10, and 15 % greatly weakened it in 3 days.

The experiments, herein recorded, were begun at the suggestions of Dr. DUGGAR and a large part of the work was done in the Missouri Botanical Garden, St. Louis in U.S.A. during a period from October, 1925 to June, 1926, while additional experiments were carried out in the Botanical Institute of the Hokkaido Imperial University. The writer wishes to express his sincere gratitude to Dr. K. MIYABE, Dr. B. M. DUGGAR and Dr. S. ITO for their helpful advices and kindly criticisms, and to Dr. G. T. MOORE for the use of facilities of the Missouri Botanical Garden.

Material and method

Preparation of the inocula. A known weight of fresh, mosaic tobacco leaves were cut up and ground in a mortar, adding an equal weight of distilled water, until the leaf tissue was thoroughly crushed, then the juice was filtered off through two thicknesses of cheese cloth, and through a coarse filter paper on a Buchner funnel. To 5 c.c. of the filtrate in a small beaker, was added 3 c.c. of distilled water which contained 0.08, 0.16, 0.24 gm. etc., of the applied chemical, thus making the concentration of the latter in the solution 1, 2, 3% etc., respectively. For the chemical substances which are hardly soluble in water, 70% alcohol was used as the solvent and 3 c.c. of the alcoholic solu-

tion, thus obtained, was added to 5 c.c. of the mosaic juice as above. The alcoholic strength in such solution being less than 30% it exerted no appreciable effect on the virus under the condition of the writer's experiments. The material thus prepared was kept in a tightly plugged sterile test tubes under the room temperature ranging 20°C. to 26°C.

Inoculated plants. The plants used for the inoculation were vigorously growing young tobacco of unnamed variety for the experiments in the Missouri Botanical Garden, and Bright Yellow variety for those in the Hokkaido Imperial University, with stems about 2 to 3 inches high, that is 3 months to 4 months old in 5-inch pots. In the early experiments, however, it was necessary to use somewhat older plants owing to deficiency of young ones.

The applied chemicals. The chemical agents used in the experiments are as follows: Aconitine potent Merck, $C_{34}H_{47}NO_{11}$; atropine Merck, $C_{17}H_{23}NO_3$; brucine, $C_{25}H_{26}O_4N_2 + 4H_2O$; caffeine Merck, $C_8H_{10}N_4O_2 + H_2O$; nicotine Merck, $C_{10}H_{14}N_2$; piperine Merck, $C_{17}H_{19}NO_3$; quinine Merck, $C_{20}H_{24}O_2N_2 + 3H_2O$; atropine sulphate Merck, $(C_{17}H_{23}NO_3)_2SO_4H_2$; hyoscyamine sulphate, $(C_{17}H_{23}NO_3)_2SO_4H_2$; morphine sulphate Merck, $(C_{17}H_{19}NO_3)_2 \cdot SO_4H_2 + 5H_2O$; strychnine sulphate, $(C_{21}H_{22}N_2O_2) \cdot SO_4H_2 + 5H_2O$; scopolamine hydrobromide Merck, $C_{17}H_{21}NO_4 \cdot HB + 3H_2O$; amygdalin Merck, $C_{20}H_{27}NO_{11} + 3H_2O$; digitalin Merck, $C_{35}H_{56}O_{14}$; phloridzin Merck, $C_{21}H_{24}O_{10} + 2H_2O$; salicin Merck, $C_{13}H_{18}O_7$; saponin Merck (purified), $C_{32}H_{52}O_{17}$ (?); oil of geranium, oil of peppermint, oil of bergamot (artificial), and oil of mustared Merck (true distilled).

Technique of inoculation. The method of inoculation employed in this investigation was principally same as that described by DUGGAR and KARRER (8). Three inoculations were made on each plant, one near the growing tip, another at the base of a young leaf, and the other farther down the stem near the ground. The inoculum kept in a small test tube was well shaken before using to mix the precipitate in the solution with the upper supernatant fluid and poured into a small sterile beaker. Then a large drop of the inoculum was placed with a sterile scalpel on the desired spot of the stem, and with a sterile needle about 30 pricks were made through the fluid, thus working the latter into the tissue. Between different inoculations the needle was flamed, then dipped in alcohol and burned off, while the hands were washed with a dilute

formaldehyde solution and rinsed with water. All the inoculations were performed in the late afternoon and then the floor of the green house was watered so as to prevent a too rapid drying of the inoculated surface. The inoculated plants were kept under observation more than a month after inoculation and sometimes until they bloomed as far as the mosaic symptom was not manifest on the leaves, because it had been found that some of the plants inoculated with the inactivated virus had failed to show the symptom on the leaves but shown on the petals.

Experimental results

The results of experiments will be shown summarized in the following tables.

Table 1. Effects of various alkaloids on the infectivity of the mosaic tobacco juice

alkaloid	date of inoculation	concentration	duration of treatment	No. of inoculated plants	No. of affected plants	percent of infection
aconitine	Dec. 3, '25	1 %	3 days	10	9	90
"	May 1, '26	2* %	6	20	19	95
atropine	Feb 11, '26	1/4	3	10	10	100
"	"	1/2	"	10	8	80
"	Oct. 6, '29	1	1	10	8	80
"	Dec. 3, '25	"	3	10	2	20
"	Dec. 31, '25	"	"	10	1	10
"	Feb. 11, '26	"	"	10	0	0
"	Sept. 9, '29	"	"	10	0	0
"	Oct. 6, '29	2	1	10	2	20
"	Dec. 31, '25	"	3	10	0	0
"	Sept. 9, '29	"	"	10	0	0
"	May 1, '26	"	5	20	0	0
brucine	Dec. 3, '25	1	3	10	9	90
"	Mar. 25, '26	"	5	10	10	100
"	May 1, '26	2	6	20	12	60
caffeine	Oct. 21, '25	1	2	10	10	100
"	Dec. 31, '25	"	3	10	10	100
"	Dec. 14, '25	"	5	9	8	89
"	Oct. 21, '25	2*	2	10	10	100
"	Dec. 31, '25	"	3	10	9	90
nicotine	Oct. 6, '29	1	1	8	7	88
"	Sept. 9, '29	"	3	10	5	50
"	Nov. 17, '25	"	5	9	3	33
"	Mar. 3, '26	"	"	10	4	40
"	Mar. 25, '26	"	"	10	10	100

alkaloid	date of inoculation	concentration	duration of treatment	No. of inoculated plants	No. of affected plants	percent of infection
nicotine	May 27, '26	1 %	5 days	20	18	90
"	Oct. 6, '29	2	1	10	8	80
"	Sept. 9, '29	"	3	10	2	20
"	Mar. 3, '26	"	5	10	2	20
"	May 1, '26	"	"	10	1	10
"	May 27, '26	"	"	20	10	50
"	May 30, '26	3	3 hours	20	8	40
"	Oct. 6, '29	"	1 day	10	2	20
"	Sept. 9, '29	"	3	10	1	10
"	May 27, '27	"	5	20	0	0
"	May 18, '26	5	"	20	0	0
piperine	Dec. 3, '25	(1)	3	10	10	100
quinine	Oct. 21, '29	0.9*	3	10	6	60
"	Nov. 2, '29	"	5	20	8	40
control** (untreated juice)	Oct. 21, '25	...	...	10	10	100
"	Nov. 17, '25	...	...	9	9	100
"	Dec. 3, '25	...	...	10	10	100
"	Dec. 14, '25	...	...	10	10	100
"	Dec. 31, '25	...	...	15	15	100
"	Feb. 11, '26	...	...	15	15	100
"	Mar. 3, '26	...	...	10	9	90
"	Mar. 25, '26	...	...	10	10	100
"	May 1, '26	...	...	10	10	100
"	May 18, '26	...	...	20	20	100
"	May 27, '26	...	...	20	20	100
"	May 30, '26	...	...	20	20	100
"	Sept. 9, '29	...	...	9	8	89
"	Oct. 6, '29	...	...	10	10	100
"	Nov. 2, '29	...	...	10	10	100

*: approximately.

**: To 5 c.c. of the mosaic juice, filtered through two thicknesses of cheese cloth and through a coarse filter paper, was added 3 c.c. of distilled water or 70% alcohol.

Table 2. Effects of salts of alkaloid on the infectivity of
mosaic tobacco juice

salt	date of inoculation	concentration	duration of treatment	No. of inoculated plants	No. of affected plants	percent of infection
atropine sulphate	Apr. 8, '26	2 %	5 days	10	7	70
"	May 18, '26	5	"	20	16	80
hyoscyamine sulphate	Dec. 7, '25	1	5	10	8	80
"	Apr. 8, '26	2	"	10	9	90
"	May 18, '26	5	"	20	11	55
morphine sulphate	Oct. 21, '25	1	1	10	10	100
"	Dec. 7, '25	"	5	8	8	100
"	Apr. 8, '26	2	"	10	10	100

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salt	date of inoculation	concentration	duration of treatment	No. of inoculated plants	No. of affected plants	percent of infection
strychnine sulphate	Oct. 21, '25	1 %	1 day	10	10	100
"	Dec. 7, '25	"	5	10	10	100
"	Apr. 8, '26	2	"	10	10	100
scopolamine hydrobromide	Dec. 7, '25	1	5	10	9	90
control (untreated juice)	Oct. 21, '25	...	...	10	10	100
"	Dec. 7, '25	...	...	10	10	100
"	Apr. 8, '26	...	...	10	10	100
"	May 18, '26	...	...	20	20	100

Table 3. Effects of glucosides on the infectivity of the mosaic tobacco juice

glucoside	date of inoculation	concentration	duration of treatment	No. of inoculated plants	No. of affected plants	percent of infection
amygdalin	Oct. 21, '25	1 %	2 days	10	10	100
"	Nov. 17, '25	"	5	9	9	100
"	May 2, '25	2	"	10	10	100
"	May 5, '26	5	"	20	19	95
digitalin	Dec. 14, '25	1	3	10	8	80
"	Mar. 25, '26	"	5	10	10	100
"	Dec. 14, '25	2	3	10	8	80
"	May 1, '26	"	5	10	10	100
"	May 18, '26	5	"	20	3	15
phloridzin	Dec. 3, '25	1	3	10	10	100
"	May 2, '26	2	5	10	10	100
"	May 5, '26	3	"	20	20	100
salicin	Nov. 15, '25	1	5	9	8	89
"	May 2, '26	2	"	20	20	100
saponin	Nov. 15, '25	1	5	9	6	67
"	May 2, '26	2	"	10	10	100
"	May 5, '26	5	"	20	13	65
"	May 18, '26	10	"	20	7	35
control (untreated juice)	Oct. 21, '25	...	...	10	10	100
"	Nov. 15, '25	...	...	9	9	100
"	Nov. 17, '25	...	...	9	9	100
"	Dec. 3, '25	...	...	10	10	100
"	Dec. 14, '25	...	...	10	10	100
"	Mar. 25, '26	...	...	10	10	100
"	May 1, '26	...	...	10	10	100
"	May 2, '26	...	...	10	10	100
"	May 5, '26	...	...	10	10	100
"	May 18, '26	...	...	20	20	100

*Table 4. Effects of volatile oils on the infectivity of
the mosaic tobacco juice*

oil	date of inoculation	concentration	duration of treatment	No. of inoculated plants	No. of affected plants	percent of infection
oil of geranium	Apr. 24, '26	2 %	6 days	10	7	70
"	May 28, '26	3	"	20	11	55
oil of peppermint	Apr. 24, '26	2	6	10	8	80
oil of bergamot	Apr. 24, '26	2	6	10	10	100
oil of mustard	Apr. 24, '26	2	6	10	2	20
"	May 28, '26	3	"	20	3	15
control (untreated juice)	Apr. 24, '26	...	...	10	10	100
"	May 28, '26	...	...	20	20	100

These experiments were carried out in green houses where the temperature between 20° and 29°C was predominating. Tobacco plants inoculated with the untreated mosaic juice developed the mosaic symptom mostly 9 to 11 days after inoculation while those inoculated with the virus, partly inactivated with chemical agents showed the symptom a few days later, the average length of incubation period being 10 to 15 days.

As shown in these tables, the virus of the mosaic disease of tobacco is remarkably resistant to various kinds of alkaloid, glucoside, and etherial oil, when these chemical agents are applied to the mosaic juice. It resisted the saturated solution of aconitine (approxim. 2% or 0.03 mol. sol.) and the nearly saturated solutions of morphine sulphate (2% or 0.03 mol. sol.), strychnine sulphate (2% or 0.02 mol. sol.), salicine (2% or 0.07 mol. sol.), and phloridin (3% or 0.07 mol. sol.), for 5 days, and also brucine in concentration of 1 % (0.02 mol. sol.), digitalin of 2 % (0.025 mol. sol.), and amygdalin of the strength of 5 % (0.11 mol. sol.) for the same period. The virus resists the saturated solution of caffeine (approxim. 2 % or 0.10 mol. sol.) for 3 days or probably longer. But it is partly inactivated with brucine in concentration of 2 % (0.04 mol. sol), oil of geranium of 3 %, and hyoscyamine sulphate of 5 % (0.07 mol. sol.) in 5 or 6 days. Oil of mustard of a strength of 2 % and digitalin in 5 % concentration (0.065 mol. sol.) are effective to destroy the virus in 5 days.

Nicotine, in 1 or 2 % concentration (0.06 or 0.12 mol. sol.) seems to be considerably toxic to the virus under certain conditions and it remarkably attenuates in 3 % concentration (0.18 mol. sol.), the infectivity of the virus in 3 hours and completely destroys its virulence in 3 to 5 days. Atropine seems to be more effective to inactivate the virus, the latter being unable to resist 1 % concentration (0.035 mol. sol.) for 3 days under certain conditions and completely inactivated in 2% concentration (0.07 mol. sol.) of this alkaloid in 1 to 3 days. *Atropa Belladonna* L. has been reported by ALLARD (1) and also by DICKSON (7) to be immune to the mosaic disease of tobacco. Whether the immunity of this plant can be attributed partly to its atropine content is uncertain, because of the small amount of this alkaloid contained in this plants (18). The saturated solution of quinine (nearly 0.9 % or 0.03 mol. sol.) appears to weaken the infectivity of the virus considerably in 3 to 5 days.

Saponin in concentration of 2 % (0.03 mol. sol.) shows no appreciable effect on the virulence of the virus and even in the strength of 10 % (0.15 mol. sol.) it can not completely destroy the infectivity of the virus in 5 days. In connection with this it will be interesting to note that the viruses of several animal diseases such as vaccinia, rabies, fowl pest and chicken sarcoma have been reported to be easily destroyed by saponin at comparatively dilute concentrations. According to RUSS (16) and LANDSTEINER (13), saponin in concentration of 1 or 0.5 % destroyed the virulence of the virus of fowl pest in 30 minutes. RUSS considered the virus of fowl pest to be of "tierischer Natur", because saponin showed no deleterious effect on certain bacteria such as *Vibrio Cholerae*, *Bac. typhi*, *Bac. anthracis*, and *Staph. pyogeneus* and yeast, while it was injurious to both *Trypanosoma Lewsii* and the virus of fowl pest. (Prior to him NEUFELD and PROWAZEK (14) pointed out the fact that saponin has toxic effects upon *Trypanosoma* and *Spirochaeta* although it is harmless for bacteria.) EISLER (9) states that the virus of rabies was destroyed by 0.25 or 0.5 % saporin in 4 to 6 hours. The virus of chicken sarcoma, according to ROUS and MURPHY (15), is destroyed by saponin in strength greater than 1:800 (0.125%) within 2 hours. The virus of vaccinia is also inactivated by saponin in 0.5 % strength in 30 minutes, and in 0.05 % concentration in 18 hours, as reported by FRIEDBERGER and YAMAMOTO (10).

Summary

The virus of the mosaic disease of tobacco was remarkably resistant to various kinds of alkaloid, salt of alkaloid, glucoside, and etherial oil, when it was subjected to these chemical agents in the mosaic tobacco juice.

It resisted the saturated solution of aconitine (approxim. 2% or 0.03 mol. sol.), and the nearly saturated solutions of morphine sulphate (2% or 0.03 mol. sol.), strychnine sulphate (2% or 0.02 mol. sol.), salicin (2% or 0.07 mol. sol.), and phloridzin (3% or 0.07 mol. sol.) for 5 days, and also brucine in concentration of 1 % (or 0.02 mol. sol.), digitalin in concentration of 2 % (0.025 mol. sol.) and amygdalin of the strength of 5 % (or 0.11 mol. sol.) for the same period.

Oil of mustard of the strength of 2% and digitalin in 5 % concentration (0.065 mol. sol.) were effective to destroy the virulence of the mosaic juice in 5 days.

Nicotine in 1 or 2 % concentration (0.06 or 0.12 mol. sol.) were considerably toxic to the virus under certain conditions, while, in 3 % strength (0.18 mol. sol.) it remarkably attenuated the infectivity of the mosaic juice in 3 hours and completely destroyed it in 3 to 5 days.

Atropine was more effective to inactivate the virus, the latter having been unable to resist it in 1 % strength (0.035 mol. sol.) for 3 days under certain conditions and completely destroyed in 2 % concentration (0.07 mol. sol.) of this alkaloid in 1 to 3 days.

Saponin in concentration of 2 % (0.03 mol. sol.) shows no appreciable effect on the virulence of the virus and even in the strength of 10 % (0.15 mol. sol.) it could not completely destroy the virus in 5 days.

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摘 要

煙草のモザイク病の病源体に対するアルカロイド、配糖体及揮發油の影響を知らむとして、之等の藥品の一定量を病葉の汁液に加へ、一定時間後に之を健全なる煙草苗に接種して其發病率を求めたり。實驗に供したる藥品は、アコニチン、アトロピン、プルシン、カフェン、ニコチン、ピペリン、キニン、硫酸アトロピン、硫酸ヒオシアミン、硫酸モルフィン、硫酸ストリキニン、臭化水素酸スコボラミン(以上アルカロイド及其鹽類合して十二種)、アミグダリン、デギタリン、サリシン、サボニン、フロリジン(以上配糖体五種)、ゼラニウム油、薄荷油、ベルガモット油、芥子油(以上揮發油四種)、の二十一種なり。

ヴァイラス(virus)が之等の藥品に対する抵抗力は甚強く、アコニチンの飽和液(約二パーセント即約0.03モル液)、硫酸ストリキニン、サリシン及フロリジンの飽和に近き液(硫酸ストリキニン二パーセント即約0.02モル液、サリシン二パーセント即約0.07モル液、フロリジン三パーセント即約0.07モル液)の中にありて五日間其感染力を害せられず。プルシンの一パーセント液(約0.02モル)デギタリンの二パーセント液(約0.025モル)、アミグダリンの五パーセント液(約0.11モル)、も五日間にヴァイラスの毒性を減ずる能はず。

芥子油は二パーセント、デギタリンは五パーセント(約0.065モル)の濃度に於て五日間にヴァイラスの毒性を著しく弱む。

ニコチンは一乃至二パーセント(約0.06-0.12モル)の濃度にて三日間にヴァイラスの感染力を弱むるこゝあり。三パーセントの濃度にては三時間にしてヴァイラスの毒性を半減せしめ、三乃至五日間に之を全く奪ふ。

アトロピンはヴァイラスに對し更に有害にして、ヴァイラスは其一パーセント液(約0.035モル)に三日間抵抗し得ざるこゝあり。二パーセントの濃度にては一乃至三日にて其感染力を全く奪ひ去らる。

サボニンは二パーセントの濃度(約0.03モル)にてはヴァイラスに五日間作用せしむるも更に影響なく、十パーセントの濃度に於てすら五日間にヴァイラスの毒性を完全に奪ふを得ず。恐水病、牛痘、鶏のベスト、鶏の sarcoma のヴァイラスはサボニンの0.5パーセントの濃度にて三十分乃至數時間内に其毒性を全く失ふに比し、煙草のモザイク病のヴァイラスがサボニンに對して斯の如く抵抗力大なるは注目し價す。