EXPERIMENTAL CULTIVATION OF ERGOT AT JORHAT, ASSAM AND EXTRACTION OF ALKALOIDS

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ABSTRACT

Climatic conditions of Jorhat, a high percentage of relative humidity and low termperature during January, February are highly favourable for the infection and yield of ergot at Jorhat. The best time of sowing rye is the first fortnight of October and artificial infection during January. Out of 15 strains of *Claviceps purpurea* used in this study, the "ergometrine" strain and "ergotamine" strain, received from the University of Johannes, Gutenberg, Mainz, performed most efficiently under the condition at Jorhat, giving high yields of sclerotia and alkaloids. The sclerotia produced by these strains were also larger in size than the average.

Artificial infection with spore suspension in cane-sugar (4·5%) produced higher yields of sclerotia of larger size as compared with artificial infection with spore suspension in water. A method for the extraction of ergotamine and ergometrine from the sclerotia is described. Following this method of extraction, the yield of ergometrine and ergotamine obtained could be favourably compared with the yields reported by other workers. Good prospect of large-scale cultivation of ergot under the climatic conditions of Jorhat and extraction of alkaloids is indicated by the study.

INTRODUCTION

PRODUCTION of ergot by artificial infection of rye (*Secale cereale* L.) with *Claviceps purpurea* (Fr.) Tul. has been attempted in India since 1942. Particular mention may be made of the work done in the Nilgiris (Marudarajan *et. al.*, 1950), in the Rongo Hills of Darjeeling District (Biswas, 1955–59), and at Jammu and Kashmir (Gandotra and Ganguly, 1962). While the work both at Nilgiris and Rongo Hills is reported to have been discontinued, the total acreage and production at Jammu during 1967 was

reported to be 77 acres and 1,320 Kg. of ergot (*CSIR News*, Vol. 17, No. 16' August 1967). However, the total production so far falls much short of the estimated annual requirement of about 50 tonnes of ergot in the country, a substantial portion of which is still met by imports.

One of the main difficulties encountered in reaching within a short time an annual production to the level of the actual requirement in the country appears to be an uncertain and erratic per acre yield of ergot in large-scale cultivations attempted so far. As per available indications, the erratic or low yields in a locality owe particularly to the uncertain or unfavourable weather conditions. It is, therefore, necessary to select suitable locations in India based mainly on climatic conditions for the large-scale cultivation of ergot.

Reporting on the natural development of rye-ergot in the USSR, Vladmirsky (1939) observed that the development of ergot was the highest in regions with a relative humidity of 74 per cent and above and a temperature below 15° C. The development was less in regions with a relative humidity of 60 per cent and was strikingly reduced in regions with a relative humidity of 55–58 per cent. Hynes (1941) working on ergot production in New South Wales, Australia, observed that a project on commercial cultivation of ergot should be considered only in cooler and moister localities. Melville (1941) also suggested that the cultivation of ergot should be attempted only where the climate is sufficiently humid. Working at Jammu, Gandotra and Ganguly (1962) obtained good yields of ergot by carrying out the artificial infection during January–February when the relative humidity was above 75 per cent and the minimum temperature below 10° C. However, at Jammu the high relative humidity during January–February is the direct result of winter rains and if the rain fails or is delayed, a high humidity during the period is not maintained.

Jorhat is situated within a climatic zone characterised by a high and well-distributed rainfall, high humidity and a moderate temperature. The climatic conditions of Jorhat being apparently favourable, a study was undertaken to find out the prospects of ergot production in this region. Extraction of ergotamine and ergometrine on a laboratory scale was also undertaken by a method standardised here.

MATERIALS AND METHODS

A critical study was made of the weather data for Jorhat, particularly rainfall, relative humidity and temperature for 3 years, 1964 to 1966, and the salient climatic features favouring ergot production were noted.

In a test conducted for determination of the optimum period for sowing and artificial infection of rye at Jorhat, uniform plots of 18·6 sq. m. each were sown with rye at 10 days' intervals between October 4, 1964 and December 3, 1964. Prior to sowing, soil analysis of the plots was done and N, P and K were applied to maintain a uniform nutritional level of N_{20} P_{20} K_{20} per acre in all plots. A variety of rye, known as Ootacamund variety, originally obtained from the Nilgiris and acclimatised at Jammu, and an isolate of *Claviceps purpurea* originally isolated from ergot sclerotia obtained from Rongo Hills (Culture No. 22, RRL, Jorhat) were used for the study. Inoculum for artificial infection was prepared by growing the fungus on boiled rye and wheat for 6 weeks at a laboratory temperature of 16° C. to 20° C. and homogenising the mass in an electric blender. This was diluted 8 times with water and used for artificial infection by spraying with a Knapsack sparyer. The inoculum contained approximately 8,000 spores per c.c. Altogether six artificial infections were carried our per plot every third day in the morning beginning from the initiation of flowering of rye.

In the second test, fifteen strains of *Claviceps purpurea*, particulars and sources of which are given below, were used for artificial infection.*

RRL 1: Isolated from sclerotia growing on an unidentified grass in Kulu valley, 1962.

RRL 2: Isolated from *Triticum sativum*, received from Cifferi (Italy), 1951.

RRL 3: Strain Tyler 15 B received from Tyler, 1959, isolated from ergot of *Agropyron semicostatum*, native of Japan, 1956.

RRL 4: Strain V. Beymo (Hollard), 1952, isolated from *Secale coronatum*.

RRL 7: Strain Portugal, 1951.

RRL 10 Strain 4399, received from de Tempe, 1945. The strain is of Spanish origin but had one or two passages through rye in Holland.

RRL 11: Strain 4401, de Tempe, 1945.

RRL 12: Strain 4402, de Tempe, 1945.

* Receipt of strains 1–13 from Director, Central Bureau Voor Schimmel cultures, Baarn (Holland); 15 from Director, CMI, England; 16 and 23 from Prof. G. Gjerstad, Director, Alkaloid Biosynthesis Research, University of Texas, USA; 22 from Director, Medicinal Plants, West Bengal and 27 and 28 from Prof. H. Rochelmeyr, Johannes Gutenberg University of Mains is gratefully acknowledged by the authors.

RRL 13: Strain 4403, de Tempe, 1945.

RRL 15: Received from Commonwealth Mycological Institute, Surrey, England (IMI 44613).

RRL 16: "Gjerstad" strain named after Dr. G. Gjerstad of University of Texas, USA. Isolated from sclerotia on rye originally received from M/s. Lilly Company of Indianapolis, Indiana.

RRL 22: A mutant of an isolate originally received from Rongo Hills, West Bengal.

RRL 23: Clavine alkaloid producing strain received from the University of Texas.

RRL 27: High ergometrine producing strain received from Pharmaceutical Academy, Johannes-Gutenberg University, Mainz.

RRL 28: High ergotamine producing strain received from Pharmaceutical Academy, Johannes-Gutenberg University, Mainz.

A plot of 18·6 sq. m. of rye was infected by each of the strains. On the basis of the data obtained in the first test for the best time of sowing and artificial infection of rye, sowing was done in the first week of October and artificial infection between the 1st and 3rd week of January. The variety of rye and the method of artificial infection were the same as in the first test.

In the third test artificial infection was carried out with spore suspension prepared in 4·5 per cent cane-sugar solution and compared with spore suspension without cane-sugar with a view to find out confirmation or otherwise of an earlier finding by Gandotra and Ganguly (1962) that artificial infection with spore suspension prepared in 5·0 per cent cane-sugar solution resulted in a higher yield of ergot. The two strains RRL 27 and RRL 28, obtained from University of Johannes, Gutenberg, Mainz, were used for this study.

Observations were taken on (1) percentage of infected earheads (5 samples of 0·2 sq. m. each), (2) yield of ergot per plot of 18·6 sq. m. in the 1st and 2nd test and 26·9 sq. m. in the 3rd test, (3) measurement of sclerotia. Samples of sclerotia produced by using various strains of *Claviceps purpurea* in the tests were chemically analysed and the percentages of total and water-soluble alkaloids present in the samples were noted following the method prescribed in the *British Pharmaceutical Codex*, 1963.

A study was made on the probable relationship between the morphological and cultural characteristics of various strains of *Claviceps purpurea* and their efficiency in ergot production on rye. The 15 strains used in the present study were grown in PDA and Oatmeal agar and five replicates of each strain were maintained in each medium. Observations were taken 30 days after inoculation of the medium on colour and diameter of colony, nature of growth (aerial or submerged), colour, septation and width of hyphae, amount of sporulation and shape and size of conidia. For estimation of the amount of sporulation, 5 ml. of sterile distilled water was poured in each flask (250 ml. Erlenmeyer flask), the surface of the medium scraped and mixed with the water. Drops of suspension were examined under microscope, the number of conidia per unit area were counted and the data expressed as the number of conidia per ml. of the suspension.

For extraction of ergometrine and ergotamine from the sclerotia strain Nos. RRL 27 and 28 were used. 500 gm. of sclerotia, ground to fine powder (100 mesh) was soaked in 100 ml. dilute ammonia and flooded with a 5-fold solvent mixture of chloroform and alcohol in 9 : 1 ratio. The mixture was shaken for one hour. A clear extract was obtained by filtration taking care to prevent the loss of solvent by evaporation. Two washes with small volumes of solvent mixture were necessary to extract the alkaloids completely. The last trace of chloroform was displaced from the meal by water. The combined chloroform extract was evaporated under vacuum at a temperature not exceeding 40° C. to 200 ml. 100 gm. of a cation exchange resin was taken in a flask containing 500 ml. of 1·0% tartaric acid solution to which the chloroform extract was added. The flask was then stirred for 2 hours at the end of which nearly the entire amount of alkaloids was absorbed by the resin. The resin was filtered off and washed with deionised water, ethanol and water.

The resin was packed into a 3 cm. wide column with a light suction. The column was first eluted with 200 ml. of 10% solution of sodium chloride containing 1% ammonia at a flow rate of 5–10 ml. per minute and finally with water. The water-soluble alkaloid in the effluent was basified with ammonia and extracted with chloroform. The alkaloid in the chloroform phase was reacted with maleic acid and maleate of ergometrine was crystallised, m.p. 191–192° C.

The ion exchanger was then eluted with ethanolic chloroform at a flow rate of 5–6 ml./minute till no further alkaloid was extractable.

The chloroform solution was taken in 1% tartaric acid solution and the chloroform evaporated. Ergotamine tartarate was then crystallised from methanol, m.p. 203° (decomp.).

Further identification of the two products was done by paper chromatography following the method of Jolan Tuzson *et al.* (1958).

All operations were done with the exclusion of light as far as practicable.

RESULTS

A study of the weather data for Jorhat for 3 years (1964–66) showed that (1) during the period from the first week of January to the third week of February, the mean percentage of relative humidity was well over 70%; (2) the total rainfall during the two months, January and February, was 24·1, 56·5 and 52·9 mm. respectively for 1964, 1965 and 1966 and the total number of rainy days during the corresponding periods was only 5, 8 and 9 respectively; (3) the minimum and maximum daily temperatures during the first week of January to the third week of February were below 15° C. and 25° C. respectively.

Thus it may be seen that during the period from the 1st week of January to 3rd week of February the relative humidity was well over 70 per cent and minimum temperature below 15° C. a combination of factors highly favourable for ergot infection. It is significant to note that the amount of rainfall and the total number of rainy days during this period were quite inadequate to directly account for a continuous high humidity during this period. The high humidity during January and February at Jorhat appears to be the result of a predominantly foggy and cloudy weather which is independent of rainfall and a high relatiye humidity is maintained during most of this period when there is little or no rain.

The weekly averages for percentage of relative humidity, minimum and maximum temperatures and rainfall for the months of January and February for 3 years (1964–1966) are presented in Fig. 1.

Data on the time of sowing and artificial infection of rye are presented in Table I.

It appears from the data that sowing rye in the first week of October and artificial infection between 2nd and 21st of January gave the best results and both the percentage of infection and yield of sclerotia progressively declined with subsequent sowings and infections. An apparent reduction in the size of sclerotia with later infections is also indicated. Thus, sowings in the

first fortnight of October and artificial infection during January appear to be optimum under the conditions at Jorhat. Artificial infections later than January reduced the yield of sclerotia.

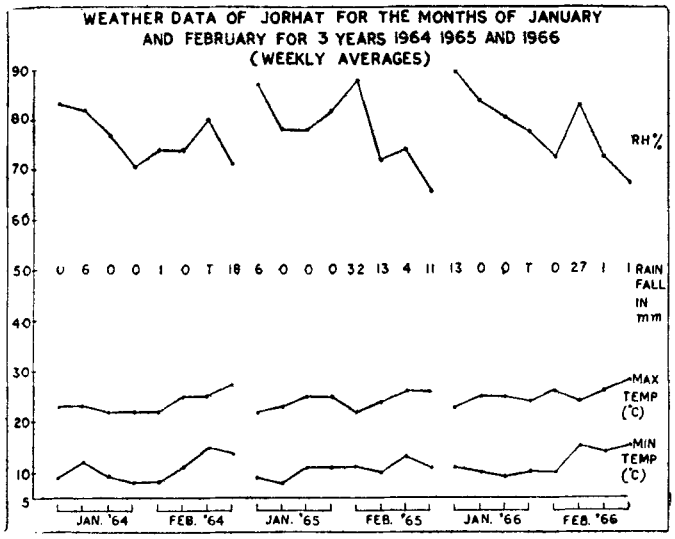

FIG. 1

TABLE I

Percentage of infected earheads (average of 5 samples of 0·2 sq. m. each), yield of ergot sclerotia in gm. per plot (18·6 sq. m.) and size of sclerotia in relation to period of sowing and artificial infection of rye

Date of sowing	Period of artificial infection	Percentage of infected earheads	Yield of ergot sclerotia in gm. per 18·6 sq. m.	Length × dia. of sclerotia in cm. (mean values)
4-10-1964	2-1-1965 to 21-1-1965	86·5	187	2·90×0·28
14-10-1964	12-1-1965 to 24-1-1965	82·4	172	2·62×0·26
24-10-1964	14-1-1965 to 25-1-1965	80·0	150	2·40×0·24
3-11-1964	15-2-1965 to 20-2-1965	78·9	124	2·01×0·22
13-11-1964	25-2-1965 to 3-3-1965	72·5	81	2·19×0·26
23-11-1964	2-3-1965 to 21-4-1965	60·5	74	2·00×0·21
2-12-1964	14-3-1965 to 28-4-1965	42·5	53	1·97×0·20

Data for the 2nd test are presented in Table II.

TABLE II

Percentage of infection, and yield, alkaloid content, and size of sclerotia produced by various strains of Cleviceps purpurea*

Strain No.	Percentage of infected ear heads	Yield of sclerotia in gm. per plot (18·6 sq.m.)	Calculated yield in kg. per acre	Percentage of total alkaloids	Percentage of water-soluble alkaloids	Length × dia. of sclerotia in cm. (mean values)
RRL 1 ..	31·7	70·6	15·3	0·38	0·020	2·0×0·40
RRL 2 ..	18·4	19·2	4·2	0·29	0·009	1·8×0·32
RRL 3 ..	36·4	82·9	18·0	0·28	0·011	2·2×0·43
RRL 4 ..	39·0	82·5	17·9	0·15	0·006	2·1×0·42
RRL 7 ..	69·7	211·0	45·9	0·29	0·005	2·8×0·48
RRL 10 ..	32·0	53·3	11·6	0·41	0·007	2·2×0·36
RRL 11 ..	48·2	86·5	18·8	0·47	0·017	2·9×0·5
RRL 12 ..	46·6	89·5	19·4	0·26	0·006	2·8×0·48
RRL 13 ..	36·2	75·9	16·5	0·36	0·015	2·6×0·48
RRL 15 ..	27·6	44·1	9·6	0·32	0·012	2·6×0·46
RRL 16 ..	39·9	83·0	18·0	0·20	0·005	2·8×0·46
RRL 22 ..	0·03	1·2	0·2	0·11	0·001	1·8×0·48
RRL 23 ..	0·2	2·4	0·5	0·16	0·004	2·2×0·43
RRL 27 ..	96·5	568·0	123·7	0·50	0·040	3·9×0·54
RRL 28 ..	92·0	535·5	116·6	0·43	0·008	3·0×0·52

* B.P. Standard for percentage of alkaloids in ergot sclerotia—0·15 per cent total alkaloids and 0·01 per cent water-soluble alkaloids.

A wide range of variation in the performance of the various strains has been noticed. Of all the strains used, strain No. RRL 27, the "ergometrine" strain received from Pharmaceutical Academy of the University of Johannes-Gutenberg, Mainz, was found to be the best in every respect, *i.e.*, infectivity on rye, yield of ergot and alkaloid content particularly the water-soluble alkaloid (ergometrine). The sclerotia produced by this strain were larger in size than those produced by other strains. The next best strain as regards the yield of ergot was the "ergotamine" strain (RRL 28) also

received from the University of Johannes-Gutenberg, Mainz. Yields of sclerotia produced by other strains were lower. So far as the total alkaloid is concerned sclerotia produced by all the strains except RRL 22 conformed to the standard. However, only 6 strains, RRL 1, 3. 11, 13, 15, and 27 were above standard as regards water-soluble alkaloids.

Data for the third test are presented in Table III.

TABLE III

*Variation of yield, weight, alkaloid content and size of sclerotia due to inoculum with 4·5% cane-sugar**

Strain No.	Treatments	Yield of sclerotia in gm. per plot 64·1 sq. m.	Mean wt. of 100 sclerotia in gm.	Percentage of total alkaloids	Percentage of water-soluble alkaloids	Length × dia. of sclerotia in cm. (mean values)
1	2	3	4	5	6	7
RRL 27	Inoculum with sugar	1830	10·52	0·438	0·054	4·6×0·58
	Inoculum in sterile water	1610	8·62	0·436	0·047	3·9×0·57
RRL 28	Inoculum with sugar	1735	10·95	0·452	0·019	4·0×0·59
	Inoculum in sterile water	1565	8·85	0·434	0·009	3·8×0·56

* B.P. Standard for percentage of alkaloids in ergot sclerotia—0·15% total alkaloids and 0·01% water-soluble alkaloids.

It appears from Table III that artificial infection carried out with spore suspension in 4·5% cane-sugar produced higher yield of sclerotia, the sclerotia were larger in size and the mean weight of 100 sclerotia was higher. A slight increase in the alkaloid content was also indicated.

A considerable variation was noticed among the morphological and cultural characters of various strains, particularly the amount of sporulation and size of conidia. While a direct correlation between any single character and the ergot yielding capacity of a strain could not be established, it was observed that high ergot-yielding strain had also a high rate of sporulation and conidia of larger size. The strains RRL 27 (ergometrine strain) and RRL 28 (ergotamine strain) which gave much higher yield of ergot

than other strains had a rate of sporulation of 30,000 and 18,000 conidia per ml. respectively and the average size of conidia as $15 \cdot 0 \times 4 \cdot 5 \mu$ and $10 \cdot 0 \times 3 \cdot 0 \mu$ respectively, whereas the rate of sporulation was between 2,400 and 8,400 conidia per ml. and the size of conidia ranged between $2 \cdot 0$–$8 \cdot 5 \times 1 \cdot 2$–$6 \cdot 0 \mu$ in the rest of the strains.

The yields of ergometrine and ergotamine, the two most therapeutically important ergot alkaloids recovered from the sclerotia of the "ergometrine" strain (RRL 27) and "ergotamine" strain (RRL 28) respectively adopting the method of extraction reported in the present study are given in Table IV.

TABLE IV

Yield of ergometrine and ergotamine from sclerotia of strain No. RRL 27 *and* RRL 28 *(expressed as percentage of the weight of sclerotia)*

Strain	Ergometrine as maleate, by analysis	Ergometrine as maleate, by extraction	Ergotamine as tartarate by analysis	Ergotamine as tartarate by extraction
RRL 27 ..	0·04	0·036	0·258	0·235
RRL 28 ..	0·008	..	0·210	0·180

The recovery of ergometrine and ergotamine as shown in Table IV was to the extent of about 90%, which can be favourably compared with the yields reported by others (Czech Patent 85, 995, 1956; Zawisza and Kuczynski, 1960).

DISCUSSION

An earlier work on ergot production (Gandotra and Ganguly, 1962) showed that ergot cultivation could be successfully carried out in the plains of Jammu at altitudes less than 300 m., and high yields of ergot with high alkaloid content could be obtained in the plains provided the climatic conditions were favourable. The present work at Jorhat which is situated at an altitude of about 200 m. in the plains of Upper Assam also showed that it is not the altitude but the climate that is the important controlling factor for ergot production. During the period from the 1st week of January to the middle of February when the artificial infection carried out at Jammu gave the best results, the relative humidity was well over 70% and the minimum temperature less than 10° C. During the corresponding period when

the artificial infection carried out at Jorhat also gave a high infection and yield of sclerotia, the relative humidity was well above 70% and the minimum temperature below 15° C. Of the two factors, humidity and temperature, the former appeared to be more important as a fall in relative humidity below 70% drastically reduced the degree of infection and yield.

While a high percentage of relative humidity is usually the direct result of rainfall, the relative humidity at Jorhat during January-February does not appear to be entirely dependant upon rainfall. The total rainfall during January-February at Jorhat is only between 24–56 mm. and the total number of rainy days during this two-month period is between 5 and 9. The high relative humidity at Jorhat appears to be due to a persistent foggy and cloudy weather and as the weather data for 3 successive years have shown, the humidity was more or less constant in all the three years. The assured humidity independent of rainfall, combined with low temperature, offers a very favourable condition at Jorhat for ergot cultivation. Significantly, the formation of honey dew after a couple of artificial infections by spraying is so profuse that it is enough to use the honey dew itself for the next 4 or 5 sprayings for a high degree of infection.

The "ergometrine" strain and the "ergotamine" strain, received from the University of Johannes-Gutenberg, Mainz, performed most efficiently under the conditions at Jorhat giving high yields of sclerotia and alkaloids. The sclerotia produced by these strains were also larger in size than the average.

Artificial infection with spore suspension in cane-sugar (4·5%) appeared to produce higher yield of sclerotia of larger size, thus confirming the earlier work of Gandotra and Ganguly (1962).

Among the various organic solvents used for extraction of ergot alkaloids, a mixture of ether and alcohol as reported earlier (Czech Patent 85, 995, 1956) was found to be most suitable for obtaining optimum yields. However, the recovery of ether after extraction was found to be low rendering the process costlier. On the other hand, extraction with chloroform was found to be economic as it was easily recoverable by displacement with water. The ion exchange method of separation was found to be efficient in separating the two alkaloids. The yield of ergotamine and ergometrine following the method of extraction reported in this communication could be favourably compared with the yields reported by other workers.

SUMMARY

Climatic conditions of Jorhat, a high percentage of relative humidity and low temperature during January–February are highly favourable for ergot cultivation at Jorhat. The best time of sowing rye is the first fortnight of October and artificial infection during January. Out of 15 strains of *Claviceps purpurea* used for artificial infection "ergometrine" strain received from the University of Johannes-Gutenberg, Mainz, is found to be a highly efficient strain under Jorhat conditions giving a high degree of infection, a high yield and alkaloid content of sclerotia. Artificial infection with spore suspension in cane-sugar (4·5%) produced higher yield of sclerotia of larger size as compared with artificial infection with spore suspension in water. Good prospect of large-scale cultivation of ergot under the climatic conditions of Jorhat is indicated.

A method for extraction of egometrine and ergotamine from ergot sclerotia is described. Yields of the two alkaloids under this method were comparable to the same obtained by other workers.

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