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Yield and Yield Attributes of Reishi Mushroom (*Ganoderma lucidum*) as Affected by Maturity Levels

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Abstract: Maturity is an important factor to determine the quality of any crops. Therefore, a lab experiment was conducted to study the effect of maturity levels on yield and yield attributes of reishi mushroom. Treatment considered as 8 maturity levels, *viz*., M_0 (control), M_1 (premature stage), M_2 (Antler stage), M_3 (Conk stage), M_4 (Conk after 10 days), M_5 (Conk after 20 days), M_6 (Conk after 30 days) and M_7 (Conk after 40 days) and 2 mushroom varieties, *viz*., Gl-7 (V_1) and Gl-4 (V_2). Result revealed that the number of fruiting body, fresh weight, stalk diameter was highest for V_2 and it took maximum days for its maturity. The maximum numbers of fruiting bodies (18.06) were found in Gl-7 at T_1 (M_1V_1), while the minimum (2.13) was in Gl-4 at T_0 (M_0V_2). Therefore, maturity level helps to get highest yield and positive yield attributes of reishi mushroom.

Key words: Reishi mushroom · Yield · Quality · Yield attributes · Maturity levels

INTRODUCTION

Reishi mushroom (*Ganoderma lucidum*) is the fruiting body of saprophytic macro fungi belong to the class of Basidiomycetes. It contains various chemical substances, including more than 119 different triterpenes and several types of polysaccharides [1]. It is using in Chinese and Japanese traditional medicine for the treatment of several diseases, likes hepatitis, hypertension, hepatopathy, gastric ulcer, arteriosclerosis, nephritis, arthritis, neurasthenia leucopenia, diabetes, anorexia insomnia, hyperglycemia, chronic bronchitis, bronchial asthma, cancer and others [2]. It is normally cultivated in solid substrates such as grains or other ligonocellulosic materials such as straw and sawdust (Stametes 2000) that support the growth and development of mycelium and fruiting bodies. Sawdust and cereal straw are the principal substrates for mushroom cultivation with the addition of supplements that substantially increase the yield [3]. There are some suitable supplements available in Bangladesh such as, sawdust, rice bran, rice straw, wheat bran, sugar cane bagasse etc. Although the economy of Bangladesh is mainly agro based and the environmental condition of it is suitable for the production of reishi mushrooms through eight months of the year in summer and rainy season. The maturity level of reishi mushroom is an important factor for the best harvesting and its medicinal values. But all the varieties of reishi mushrooms may not perform well at same maturity level. Two reishi mushroom varieties Gl-7and Gl-4 are commonly cultivated in Bangladesh. But the cultivation of reishi mushroom is just near to start in this country and the cultivators has not sufficient knowledge on its cultivation procedure, management practices and harvesting time or maturity level of fruiting body. Considering the medicinal and economical value of reishi mushroom, the experiment was undertaken to evaluate the best maturity level of reishi mushroom for the better yield and quality.

MATERIALS AND METHODS

Experimental Site and Duration: The experiment was conducted at the Biotechnology Laboratory and Culture House of National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh during July-November 2011.

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Treatments and Design of the Experiment: Eight maturity stages of reishi mushroom, *viz.,* premature stage-mycelium running completion and primodial initiation stage, antler stage-the primodial developed as fruiting body, conk stage-the fruiting body developed as conk or immature stage of pileus, 10 days after conk stage, 20 days after conk stage, 30 days after conk stage, 40 days after conk stage and control-matured fruiting body harvested after disappearance of yellow boarder on conk, as represented by M_1, M_2, M_3, M_4, M_5, M_6, M_7 and M_0, respectively were evaluated on the two varieties of *Ganoderma lucidum*, *viz.,* Gl-7 and Gl-4 as represented by V_1 and V_2. The experiment was laid out in completely randomized design with 4 replications and each replication included 16 pieces of spawn packets.

Equipments and Basic Materials: Varietiy Gl-7 and Gl-4 was collected from germplasm centre of National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh. It was cultured in biotechnology laboratory and cultivated in the culture house. Laminar air-flow transfer cabinet was used to inoculate and transfer the experimental materials in aseptic condition. It presents a gentle flow of filtered air over the cabinet and fitted with UV light for decontamination of the inner space. Ethyl alcohol (70%) was used to wipe surfaces of working areas, to rinse hands and to dip instruments, with or without subsequent flaming. It was also used to disinfect the surface of fruiting body. Although chemicals likes agar, dextrose, tetracycline, $CaCO_3$, asparagines and, organic substrate as saw dust and wheat bran were used in this experiment.

Preparation of PDA Media and Pure Culture: PDA media contain potato, dextrose, agar, aspersing and tetracycline @ 250, 20, 20, 250 and 250 g and it was used to culture for mycelium growth. To prepare one liter of PDA media, 250g of potato was measured by electric balance. Then the pills of potato were removed by knife sliced as small pieces. The pieces were boiled with one liter of water for 45 minutes and filtered by thin cloth. All the measured chemical compound of PDA media was mixed with remaining 1 L of water. Then the solutions were also boiled for 15 minutes and several times agitated with stick. After boiling, 10 ml solution was filled in each petridish and wrapped with aluminum foil. Then it was autoclaved at 121°C and 15 PSI for 45 minutes to avoid contamination. The explants (*Gonoderma lucidum*) were inoculated in contamination free environment. Laminar air flow was used to inoculate the germplasm. After sterilization the inoculums were laid in petridish using forceps by cutting with sterile scalpel. The petridish was wrapped with micro-pore under the laminar air flow and placed for pure culture preparation. After 5-10 days, the mycelium was found and used as pure culture. The pH of the medium was adjusted to 5.8 before autoclaving by adding 0.1 N NaOH. The relative humidity 80-85% and temperature 28-35°C was maintained by watering thrice a day. The light intensity was managed according to treatment and proper ventilation was also maintained in the culture house. After 18 days mycelium running was completed and used in mother culture.

Preparation of Mother Culture: Mother culture packet^{-1} was consisted of sawdust, wheat bran, water and $CaCo_3$ @ 100 g, 50 g, 150 ml and 1 g, respectively. For mother culture preparation, sawdust was used as main substrate and wheat bran as supplement. In each 300g of mother packet, above components were mixed and was filled into heat tolerant polypropylene bags of 7½ to 10½ size. The mouth of the bags were plugged by inserting water absorbing cotton and tied with rubber band after covering with brown paper. Then bags were autoclaved at 121°C and 15 PSI for 1 hour and then allowed for cooling. Each mother packet was inoculated with the pure culture at the rate of 5 square mm packet^{-1}. The relative humidity, temperature, light intensity and proper ventilation were maintained accordingly like as culture house.

Spawn Packet Preparation: The spawn packet was prepared by using sawdust and wheat bran at the ratio of 2:1. Water was added to make the moisture content 60% and $CaCO_3$ was added at the rate of 0.2% of the mixture. Polypropylene bags of 7½×10½ size were filled with 500g of substrate mixture and their mouths were plugged by inserting water absorbing cotton with the help of plastic neck and rubber. The bags were autoclaved at 121°C and 1 kg cm^{-2} for 2 hours. After autoclaving and cooling, the bags were inoculated separately with the mother culture @ 1 tea spoonful packet^{-1}. Then the packets were incubated in the incubation room for mycelial growth.

Experimental Condition: In incubation period, the inoculated packets were kept in almost dark at about 25ºC temperature. After completion of mycelium running spawn packets were opened by square sized (1×1 cm) cut on the single side middle abdomen of the packet and transferred to the culture room at 25-32ºC temperature, 85-95% relative humidity and 250-350 lux light. Water was sprayed 3 times day^{-1} and proper aeration was maintained in culture house to develop the fruiting bodies.

Data Collection and Statistical Analysis: Data on days to harvest, fresh weight, number of fruiting body, length and diameter of stalk and, diameter and thickness of pileus were analyzed and means separation were computed following Duncan's Multiple Range Test (DMRT) at 5% level of probability.

RESULTS AND DISCUSSIONS

Days to 1st Harvest and Fresh Weight: Days to 1st harvest and fresh weight showed significant variation. The variety Gl-4 required maximum days (29.78) to 1st harvest (Table 1), while it produced the lowest fresh weight (10.07 g packet^{-1}) of reishi mushroom. In case of maturity level, the maximum days (51.87) were required for 1st harvesting in T_7, while the minimum was in T_1 (Fig. 1) and the maximum fresh weight (17.34 g packet^{-1}) was in T_5 while the minimum was in T_2 (Fig. 2). It also showed significant variation in combined effect and ranged from 7.00 to 54.38 days. The highest fresh weight (21 g packet^{-1}) was in T_3V_1 and the lowest (1.69 g packet^{-1}) was in T_1V_2 (Table 1).

Days to 2nd Harvest and Fresh Weight: Days required for 2nd harvest and fresh weight was significantly influenced by varieties and maturity stages of reishi mushroom. The variety Gl-4 required maximum days (28.80) and produced the highest fresh weight (6.62 g packet^{-1}) of reishi mushroom (Table 1). The minimum days were required when harvest was done at premature stage of maturity (Fig. 1) and that time fresh weight also was minimum (Fig. 2). The maximum days for 2nd harvest (58.25) was in T_7V_2 and fresh weight (11.0 g packet^{-1}) was in T_6V_2 (Table 1).

Days to 3rd Harvest and Fresh Weight: The maximum days (28.03) were required for 3rd harvesting in V_2 and also it produced maximum fresh weight (Table 1). The maximum (54.62 days) was needed when harvest at 40 days after conk formation (Fig. 1) and fresh weight was 4.81 g packet^{-1} (Fig. 2). Third harvest and fresh weight varied significantly by their combined interaction (Table 1).

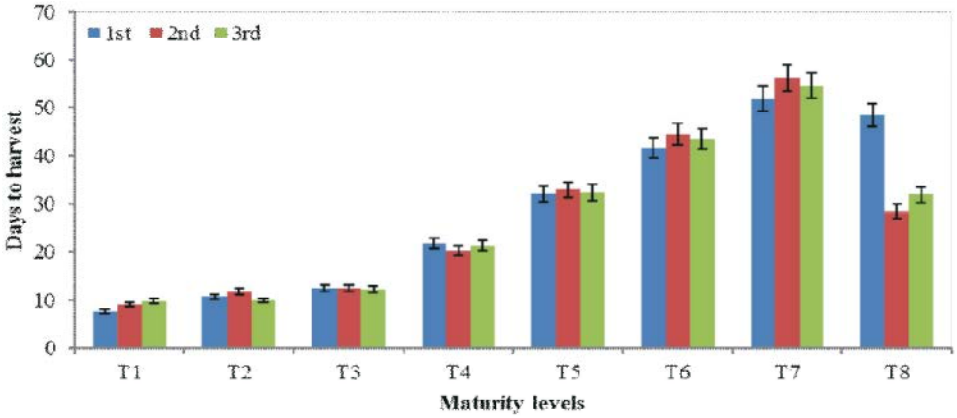

Fig. 1: Effect of maturity levels on days to harvest on reishi mushroom

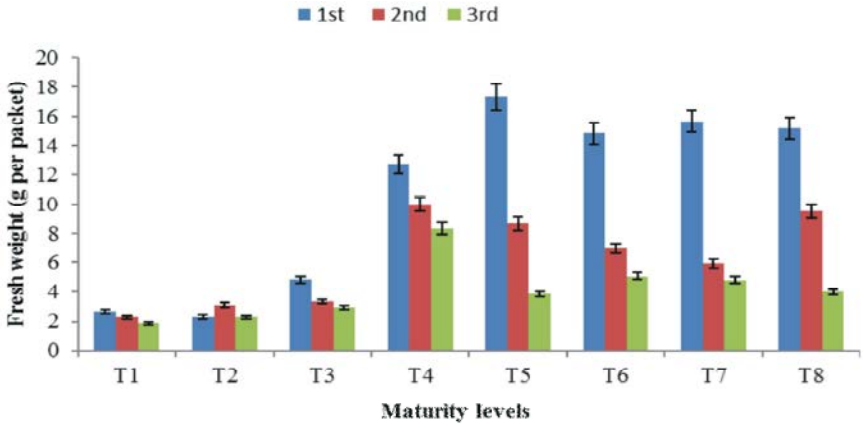

Fig. 2: Effect of maturity levels on fresh weight of reishi mushroom

Table 1: Effect of variety and maturity levels on days to harvest and fresh weight

	Days to			Fresh weight (g packet^{-1}) at		
Treatments	1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest
Variety						
V_1	26.86	25.15	25.87	11.34	5.86	3.63
V_2	29.78	28.80	28.03	10.07	6.62	4.68
Variety x Maturity levels						
M_1V_1	7.00 n	7.63 m	8.87 k	2.13 f	1.63 i	1.62 h
M_1V_2	9.94 m	11.00 l	8.25 k	1.69 f	3.12 h	1.75 gh
M_2V_1	11.75 l	12.00 k	11.50 j	3.69 ef	2.94 h	3.00 fg
M_2V_2	20.31 j	17.00 i	21.63 h	12.64 d	9.06 bcd	7.75 b
M_3V_1	30.38 h	30.50 f	30.75 g	21.00 a	9.25 bc	2.81 fgh
M_3V_2	40.88 f	43.13 d	41.75 d	17.25 b	6.06 fg	4.50 de
M_4V_1	49.38 c	54.25 b	53.50 b	17.56 b	5.00 g	3.75 ef
M_4V_2	45.25 d	25.75 g	30.75 g	14.81 bcd	9.81 b	3.87 ef
M_5V_1	8.12 n	10.63 l	10.75 j	3.19 f	2.94 h	2.12 gh
M_5V_2	11.56 l	12.63 jk	11.63 j	2.94 f	3.06 h	2.81 fgh
M_6V_1	13.19 k	12.63 jk	13.00 i	6.00 e	3.75 h	2.87 fgh
M_6V_2	23.31 i	23.75 h	21.00 h	12.88 cd	11.00 a	9.00 a
M_7V_1	51.69 b	45.75 e	45.25 c	13.69 cd	8.12 cd	4.94 cde
M_7V_2	54.38a	58.25 a	55.75a	12.44 d	7.94 de	5.69 cd
M_0V_1	33.69 g	31.25 f	32.88 f	13.81 cd	6.87 ef	5.87 c
M_0V_2	42.31 e	35.25e	34.00e	15.63 bc	9.25 bc	4.12 ef
CV (%)	2.92	2.05	2.81	16.74	12.12	20.19

In a column, means followed a common letter do not differ significantly at 0.05 level of probability, M_0 = control, M_1 = premature stage, M_2 = antler stage, M_3 = conk stage, M_4 = conk after 10 days, M_5 = conk after 20 days, M_6 = conk after 30 days, M_7 = conk after 40 days, V_1= Gl-7 and V_2= Gl-4

Number of Fruiting Body: Maturity levels were influenced significantly on the number of fruiting body. The variety Gl-4 produced the maximum number of fruiting body (7.68 packet^{-1}) and the premature stage also produced the maximum number of fruiting body (15.03 packet^{-1}), while the minimum (2.19 packet^{-1}) was in M_0 (Table 2). The highest number of fruiting body (18.06 packet^{-1}) was in the combination of M_1V_1 and the lowest number of fruiting body (2.13 packet^{-1}) was harvested from T_0V_2 (Table 2).

Length and Diameter of Stalk: To measure the length of stalk and diameter of stalk were not possible before the conk development in premature, antler and conk stage of reishi mushroom. The length of stalk was influenced insignificantly by variety and maturity levels. However, the interaction effect betwen variety and maturity level produce the stalk ranged from 0.89 to 1.69 cm (Table 2). Variety and maturity levels of reishi mushroom showed significant variation in diameter of stalk. The maximum diameter of stalk was in V_2 and M_0. It also varied significantly in combined effect and ranged from 0.38 cm in M_7V_2 to 0.63 cm in M_0V_2.

Diameter and Thickness of Pileus: To measure the diameter and thickness pileus were not possible before the conk development in premature, antler and conk stage of reishi mushroom. Diameter of pileus was significantly influenced by variety and maturity levels (Table 2). The maximum diameter (4.54 cm) was in V_1. In case of maturity level the maximum diameter (4.87 cm) was in T_7 and minimum (3.85 cm) was in T_6 level. In combined effect, the highest diameter (5.12 cm) was in T_5V_2 and the lowest (3.62 cm) in T_7V_2 (Table 2). Variety and maturity levels do not affect significantly on thickness of pileus. However, it ranged from 0.58 cm to 0.66 cm (Table 2).

Table 2: Effect of maturity levels and variety on the number of fruiting body, length and diameter of stalk, diameter and thickness of pileus

Treatments	Number of fruiting body packet^{-1}	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)
Variety					
V_1	7.68	1.45	0.461	4.54	0.66
V_2	5.47	1.33	0.477	4.130	0.58
Maturity levels					
M_1	15.03 a	-	-	-	-
M_2	11.62 b	-	-	-	-
M_3	9.56 c	-	-	-	-
M_4	4.75 d	1.03 ab	0.42 ab	4.49 a	0.58 a
M_5	3.64 e	1.54 a	0.50 a	3.88 b	0.61 a
M_6	3.37 e	1.54 a	0.40 b	3.85 b	0.58 a
M_7	2.41 f	1.51 a	0.43 ab	4.87 a	0.65 a
M_0	2.19 f	1.27 ab	0.59 a	4.60 a	0.69 a
CV (%)	8.40	17.59	17.97	9.72	11.82
Variety x Maturity levels					
M_1V_1	18.06 a	-	-	-	-
M_1V_2	15.13 b	-	-	-	-
M_2V_1	12.00 c	-	-	-	-
M_2V_2	4.63 f	-	-	-	-
M_3V_1	3.56 g	-	-	-	-
M_3V_2	3.25 gh	-	-	-	-
M_4V_1	2.56 hi	0.89c	0.40 c	4.51ab	0.63 bc
M_4V_2	2.25 i	1.54 ab	0.52 abc	3.99 bc	0.64 bc
M_5V_1	12.00 c	1.69 a	0.42 bc	4.07 bc	0.6bc
M_5V_2	8.13 d	1.49 ab	0.42bc	5.12 a	0.65 b
M_6V_1	7.13 e	1.36 ab	0.55 ab	5.03 a	0.80 a
M_6V_2	4.88 f	1.18 bc	0.45 bc	4.47ab	0.54 c
M_7V_1	3.75 g	1.54 ab	0.49bc	3.78 c	0.58 bc
M_7V_2	3.50 g	1.48 ab	0.38 c	3.62 c	0.57 bc
M_0V_1	2.25 i	1.53ab	0.44bc	4.62 ab	0.64bc
M_0V_2	2.13 i	1.19 bc	0.63 ab	4.16 bc	0.59bc
CV (%)	8.40	0.3537	17.97	9.72	11.82

In a column, means followed a common letter do not differ significantly at 0.05 level of probability, M_0 = control, M_1 = premature stage, M_2 = antler stage, M_3 = conk stage, M_4 = conk after 10 days, M_5 = conk after 20 days, M_6 = conk after 30 days, M_7 = conk after 40 days, V_1= Gl-7 and V_2 = Gl-4

CONCLUSION

The yield of reishi mushroom was greatly influenced by variety and maturity levels. The variety Gl-4 and maturity stage- conk formation after 10 days were most suitable and effective to produce fruiting body.

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Appendix 1: Different maturity levels of reishi mushroom

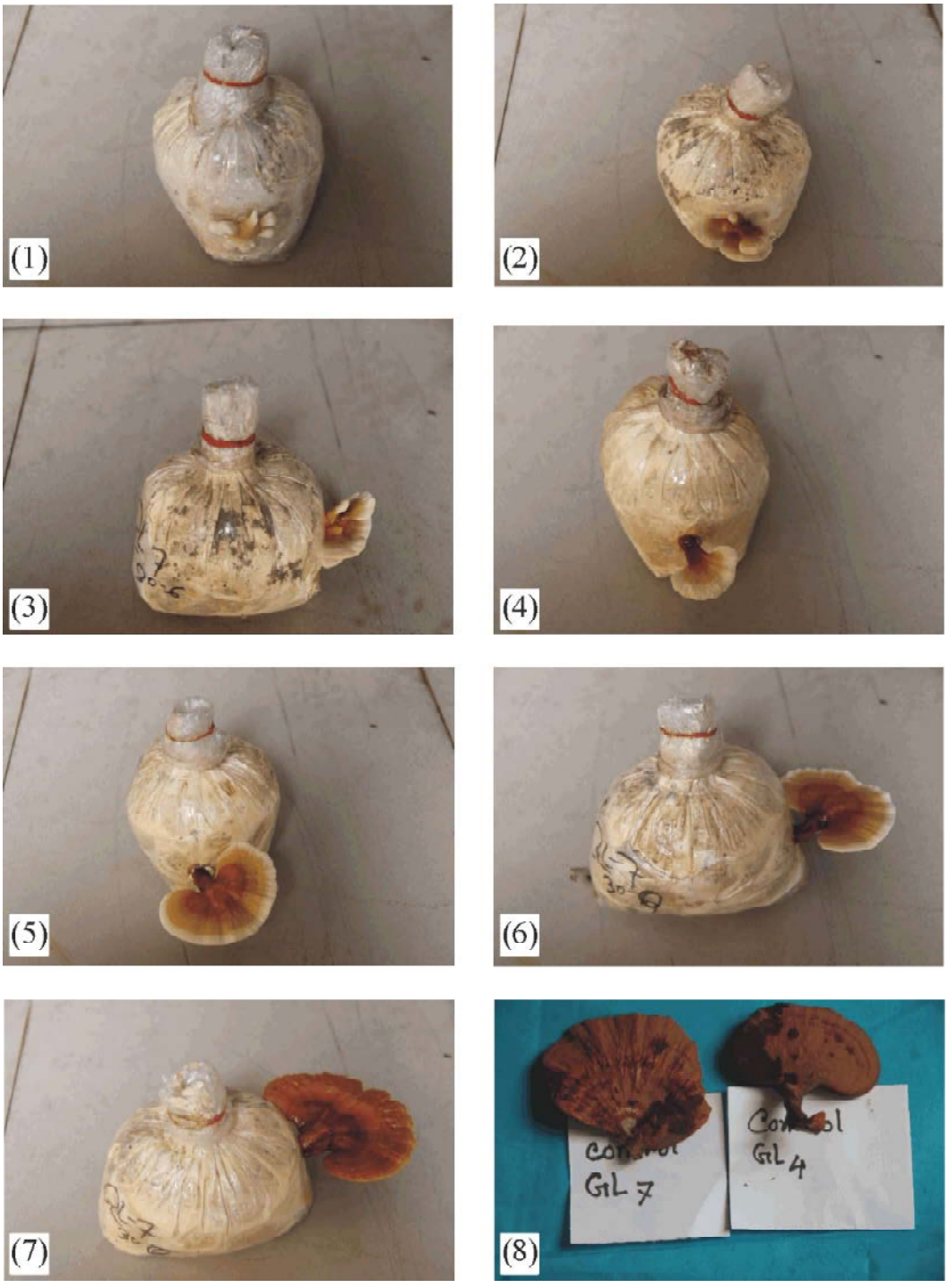

Plate: 1. premature stage, 2. Antler stage, 3. Conk stage, 4. Conk after 10 days, 5. Conk after 20 days, 6. Conk after 30 days, 7. Conk after 40 days, 8. Control stage