Model fitting for squalene content and yield. Experimental results for SQC, SQY and ERGC were analyzed by ANOVA (data in **Tables I** and **II** provided as supporting information) to test the validity of each model and to determine whether a more complex one would have a better fit. If the *F* test for the model is significant at the 5% level ($p < 0.05$), then the model can adequately account for the variation observed. If the *F* test for lack of fit is significant, then a more complicated model is needed. The full second-order polynomial model (Eq [1]) was fitted for the case of SQC and ERGC responses; since all of the factors have $p < 0.05$ (see **Table 4** of the manuscript and also **Tables I** and **II** provided as supporting information). In the case of SQY response, the full second-order model was reduced by omitting the insignificant terms X_2^2 and the interactions X_1X_2, X_1X_3, X_2X_3 ($p > 0.05$). Although X_1 appeared to have no significant linear effect to SQY, it was included in Eq [4] as its quadratic effect was significant at the 5% level (in the same tables). The models 1-3 fitted for ANOVA were found to be adequate and without significant lack of fit ($p >> 0.05$). Also, the coefficient of determination (R^2) values (0.939 - 0.976) (**Table I**) indicate a high degree of correlation between the observed and the predicted responses. Consequently, the above models can give an adequate description of the experimental data and are suitable for use in the optimization process.

Main effects of factors and interactions on SQC, SQY and ERGC responses. Eqs [2] and [4] describe the significant linear effects of the factors (X_1, X_2, X_3) tested to SQC and SQY. It is apparent from these equations that X_1 and X_3 possess significant positive effects to both SQC and SQY, indicative of a proportional relationship between these factors (terbinafine and time) and responses. The negative coefficients associated with the quadratic term for X_1 and X_3 in Eqs [2] and [4] indicate that SQC and SQY decrease when these factors approach their set maximum (+1) levels (see Experimental Design). On the other hand, X_2 affected negatively SQC and SQY. Moreover, a positive quadratic effect was noted for X_2 to SQC, while its quadratic effect to SQY was insignificant ($p > 0.05$). The significant negative interaction between X_1 and X_2 suggests an antagonistic behaviour that affects negatively SQC*,* while the positive interactions between X_1 and X_3 and also between X_2 and X_3 indicate synergism. No significant interactions were assigned to all pairs of factors regarding SQY. Coefficients estimates for model 3 and the corresponding *p* values (Eq 6, **Table I** of the supporting information) show that the two regulators X_1 and X_2 affect in an opposite way ergosterol accumulation. Thus, terbinafine enhances squalene content at the expense of ergosterol, whereas the opposite trend was found for the activity of the other regulator.

Table I. Analysis of variance of SQC, SQY and ERGC obtained using the RSM model.

Source		F value[a]	*p* value
SQC response (R^2-adj. = 0.976)			
Regression		45.31	0.000
	Linear	42.31	0.000
	Square	71.76	0.000
	Interaction	21.86	0.000
Lack of fit		2.86	0.136
SQY response (R^2-adj. = 0.939)			
Regression		17.22	0.000
	Linear	35.32	0.000
	Square	15.57	0.000
	Interaction	0.77	0.536
Lack of fit		1.27	0.401
ERGC response (R^2-adj. = 0.973)			
Regression		40.32	0.000
	Linear	64.67	0.000
	Square	21.85	0.000
	Interaction	34.44	0.000
Lack of fit		0.65	0.677

[a]F-test values are significant at the 5% level

Table II. Estimated regression coefficients and significance (*p* values) for SQC, SQY and ERGC after analysis using coded values of factors.

Term	Coefficient	*p* value
SQC response		
Constant	6.2375	0.000
Terbinafine	0.6123	0.000
Methyl jasmonate	-0.7939	0.000
Fermentation time	0.3615	0.003
Terbinafine × Terbinafine	-0.8386	0.000
Methyl jasmonate × Methyl jasmonate	0.7046	0.000
Fermentation time × Fermentation time	-0.7096	0.000
Terbinafine × Methyl jasmonate	-0.7987	0.000
Terbinafine × Fermentation time	0.3537	0.017
Methyl jasmonate × Fermentation time	0.4887	0.003
SQY response		
Constant	16.848	0.000
Terbinafine	0.086	0.886
Methyl jasmonate	-3.710	0.000
Fermentation time	4.721	0.000
Terbinafine × Terbinafine	-2.701	0.001
Methyl jasmonate × Methyl jasmonate	-0.265	0.650
Fermentation time × Fermentation time	-3.028	0.000
Terbinafine × Methyl jasmonate	-1.062	0.193
Terbinafine × Fermentation time	0.312	0.690
Methyl jasmonate × Fermentation time	-0.343	0.663

ERGC response		
Constant	2.0200	0.000
Terbinafine	-0.3532	0.000
Methyl jasmonate	0.0781	0.024
Fermentation time	0.1941	0.000
Terbinafine × Terbinafine	0.1062	0.004
Methyl jasmonate × Methyl jasmonate	-0.0777	0.022
Fermentation time × Fermentation time	0.1875	0.000
Terbinafine × Methyl jasmonate	0.2688	0.000
Terbinafine × Fermentation time	-0.2563	0.000
Methyl jasmonate × Fermentation time	-0.1237	0.009