Chapter 5

FORMULATION OF 1' – ACETOXYCHAVICOL ACETATE

5.1 Introduction

As confirmed by many experiments in Chapter 4, 1'-acetoxychavicol acetate is and interesting active substance from galanga giving the best result to control *Colletotrichum gloeosporioides* (Penz.) Sacc. mango anthracnose fruit rot disease. This substance showed even significant possibility to replace benomyl (a conventional agricultural chemical using for dipping mango fruit before export). To introduce 1'-acetoxychavicol acetate to the mango export company, a further development to be a ready-to-use product is required.

Crude extract from galanga using dichloromethane normally yields oleoresin, which contains essential oil, fat and many other organic compounds. Even after purification to get active ingredient the active substance was facing the problem of nonsoluble in water. This is the big problem of utilize 1'-acetoxychavicol acetate as dipping solution for mango. In this Chapter formulation of this active substance was studied.

According to Frei and Schmid (1996), pesticide formulation of water non-soluble substance should follow the liquid formulation technique. Nayayanan (1996) suggested the main component of liquid formulation to be 6 parts:

1) Active ingredient
2) Solvent (primary solvent, and secondary solvent, if necessary)
3) Emulsifier (primary emulsifier, and secondary emulsifier; dispersing agent and wetting agent)
4) Polymeric stabilizer (crystal inhibition)
5) Preservative (optional)
6) Others.

Government (1997) suggested the standard criteria to select the good emulsifier stability in many aspects. Mixing pesticide should retain its normal liquid condition more than 6 hrs. Bad

emulsifier usually cause the change from liquid to be cream or oily condition. In the case of good emulsifier change from liquid to be cream should not exceed 2 ml or to be oil not exceed 0.5 ml. Government (1997) also suggested Agrisol P-135 as the general emulsifier for pesticide formulation and also suggested Xylene as the solvent for a better water soluble condition.

5.2 Material and Methods

5.2.1 Screening of emulsifier and formulation studies

The experiments were conducted in collaboration with T.J.C. (Thailand) Co.Ltd., Bangkok, Thailand. Purified crystal of 1'acetoxychavicol acetate was firstly sent to start the formulation study (on 8/03/2001), and the semipurified product of L14+ L15 (more than 50% impurity) were sent on 13/07/2001 to study the possibility of using crude extract as the raw material for formulation. Triton X-100 was used as primary emulsifier, Agrisol P-135 as secondary emulsifier and Xylene as solvent. No polymeric stabilizer and preservative compound were used. The combination of different concentration yielded 5 recipes. (EC-formulation).

5.2.2 Minimum fungicidal concentration (MFC) study

Poison food technique as explained earlier using PDA mixed with different concentration of EC - formulation was used to compared the efficiency to control *Colletotrichum. gloeosporioides* (Penz.) Sacc. Effective concentration was then calculated for the application rate, when apply as dipping solution.

5.2.3 Dipping study with mango fruit

Dipping solution was produced base on the effective concentration in MFC studies, in the amount of 20 lt. Mango fruits (Nam-Dok-Mai) at the full mature stage were harvested from the plant in Chiang Mai and dipped for 5 min in the solution and dried at room temperature. The experiment was conducted in completely randomized design (CRD) with 10 replications (fruits) and compared with conventional fungicide (benomyl) 225 ppm. Disease incidence on the fruits were evaluated according to the method suggested by Korpraditsakul (1991) for 6 levels of infection.

5.3 Result and Discussion

5.3.1 Formulation of 1′-acetoxychavicol acetate

As shown in Table 5.1, which is the QC. Report information from the Company, all the 5 recipes showed a very good appearance of clearly liquid with the color pale yellow to brownish yellow depended on the amount of active ingredient, Triton X-100, Agrisol P-135 and Xylene. Only two formulates were satisfied according to the stability, i.e. recipe number 2 (crystal 1′acetoxychavical acetate 20% w/w) and recipe number 4 (L14 + L15 5% w/w).

Shafter and Bukovac (1987) reported the synergist effect of triton X-100 with active ingredient extract from plant by function as intermediate among active substance, adjuvant and others. This condition enhanced the disperse of active substance in the water when mix for spraying.

Emulsifier, Agrisol P-135 is the chemical compound in the group of N-alkyl pyrroridone. Which very beneficial as cosolvent or cosurfactant or as secondary emulsifier (Narayanan et al., 1993). These optimum condition of N-alkyl pyrroridone were the coupling of microemulsion technology with the synergy of pyrrolidones with anionic surfactant and the ability of cirtain pyrrolidone to entrance the penetration and translocation of some active thgredients, produces adjuvant system that significantly there are the efficacy of a number of pesticides when added as adjuvant to the diluted, ready-to-spray commercial pesticide formulations (Foy and Pritchard, 1996).

In the case of Xylene, Shafter *et al.* (1998) also has the synergist effect by inhibit the clean formation of active ingredient when dilute with water. Xylem also inhibit the oil formation and protect the active ingredient from oxidation activity when spray or apply on plant tissue.

5.3.2 Minimum fungicidal concentration (MFC) study

Data achieved from Chapter 4 showed the X value of 1′acetoxychavical acetate at 670 ppm. In this study concentration of active ingredient in poison food PDA were varied based on the same concentration of X = 670 ppm. and calculation was made from 20 % a.i. The results were shown in Table 5.2 , together with the correlation equation.

The effective concentration of formulation was 570 ppm (Figure 5.1), which was slightly lower than the previous MFC studies in Chapter 4 (670 ppm). Two factors suppose to be the main reason cansing this lower concentration for effectiveness. Firstly, the raw material using for formulation was crystal purified substance (84% purity), whereas raw material used in Chapter 4 was liquid (semipurified). Secondly, in the formulation there was still some other adjuvant and solvent e.g. Triton X-100, Agrisol P-135 and Xylene. These additive might enhance the effectiveness of formulation to control *Colletotrichum gloeosporioides* (Penz.) Sacc. Further studies are necessary to understand this phenomena. Stock *et al.* (1992) also reported the possibility of enhancing the efficiency of active ingredient through the synergist effect of the appropriate emulsifier and solvent.

Q.C. REPORT

No. : 009 / 44

ITEM : ~~PRODUCT OF GALINGALE~~ Date : 19 / 10 / 01

PURPOSE : To research and find the best formula

Chemical compound 's Galingale	1st arrival (8/03/44)		2nd arrival (13/07/44)		
Formulation	1%, w/w	20%, w/w	1%, w/w	5%, w/w (4.4%, w/v)	20%, w/w (17.9%, w/v)
Recipe	1	2	3	4	5
Chemical compound 's Galingale (84%) 1st	1.2	23.8	-	-	-
Chemical compound 's Galingale 2nd	-	-	1.2	6.0	23.8
Triton X-100 (Note 1)	-	3.5	-	3.5	3.5
Agrisol P-135	6.0	3.2	5.0	3.2	3.2
Xylene	93.8	69.5	93.8	87.3	69.5
TOTAL	100.0	100.0	100.0	100.0	100.0
R.T. (Initial)					
% AI. content (w/v)	-	-	-	-	-
Appearance	Cleady liquid	Cleady liquid	Cleady liquid	Cleady liquid	Cleady liquid
Colour	Pale Yellow	Brownish-Yellow	Yellow	Yellow	Yellowish-brown
Density (30° C)	——Not enough to check——		-	0.875	0.895
Emulsifiability	Good	Good	Fair	Good	Fair
Stability (2 hr.)	Good	Good	Oil 2 ml.	Good	Oil 1 ml.
(6 hr.)	Cream 3 ml.	Cream 1 ml.	Oil 3 ml.	Oil 0.5 ml.	Oil 2 ml.
After 6 hr., invert and standing 30 min.	Cream 1 ml.	Good	Oil 0.5 ml.	Good	Oil 0.5 ml.

REMARK : - Note 1 Emulsifier for this product received on 13 / 07 / 44

- Chemical compound Galingale of second time arrival did not mention % purity. (But QC. need to use its purity for calculation the formula. So. QC. used 84% purity as same as the first time arrival.)

CONCLUSION : 1. The character of Chemical compound with extracting from Galingale for two time arrival were different as follows ;

Old sample : Yellow colour New sample : Yellowish-Brown colour

2. If old sample using, the formula 1% and 20% of ai content are suitable for formulation, but the 20% formulation is more satisfactory.

3. If now sample using, the formula 5% of ai content is suitable and satisfactory for formulation.

* QC. can not test under heat test continue research because the sample is not enough. *

Chemist :	QC. Chief :	QCDM:	FM. :

Table 5.1 Q.C. Report from the Company showing the major content of 1′-acetoxychavicol acetate formulations and the conditions achieved.

Table 5.2 Poison food studies of 1′-acetoxychavicol acetate formulation to control *Colletotrichum gloeosporioides* (Penz.) Sacc.

Replication	Concentration (ppm)							
	0.25 X	0.5 X	0.75 X	X	1.25 X	1.5 X	1.75 X	X
1	54.31	76.01	86.01	84.44	90.39	90.00	94.41	97.33
2	56.04	77.81	85.37	86.74	90.05	92.23	93.76	96.01
3	55.91	75.92	84.92	89.90	88.87	87.37	90.36	94.28
4	51.83	79.37	87.14	89.95	90.11	88.56	93.01	93.06
5	53.33	74.98	86.56	88.84	87.17	91.98	89.86	96.01
6	67.64	88.43	80.10	91.05	91.99	89.92	88.70	98.53
Mean	56.15	78.75	86.10	88.47	89.87	90.01	93.35	96.37

CV = 12.91%

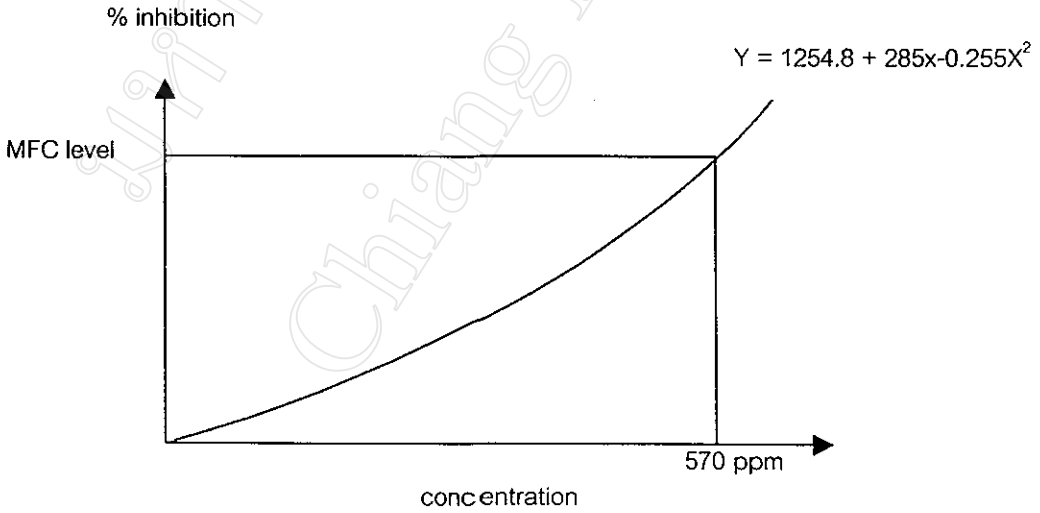

Figure 5.1 Correlation equation curve between percentage inhibition and concentration of formulation.

5.3.3 Efficiency of formulation to control *Colletotrichum gloeosporioides* (Penz.) Sacc.

Effective to control *Colletotrichum. gloeosporioides* (Penz.) Sacc. was evaluated according to the standard value used by Korpradisakul (1991) as follow :

Level 0 = no symptom
Level 1 = symptom 1-5% of overall peel area
Level 2 = symptom 6-15% of overall peel area
Level 3 = symptom 16-30% of overall peel area
Level 4 = symptom 31-50% of overall peel area
Level 5 = symptom >50% of overall peel area

Figure 5.2 showed clear effectiveness of 1'acetoxychavical acetate formulation to control anthracnose mango fruit rot disease when compared to control. Disease symptom was less than 1-5%, similar to the fruits treated with benomyl. This confirms the successful of formulation for using for postharvest mango fruit treatment, or even for other fruits like papaya, rose apple, banana etc.

As shown in Table 5.3 formulation at 570 ppm. was the most effective fungicide, followed by benomyl and control (water), respectively. In the case of control treatment many fruits showed the disease incidence up to level 5. benomyl treated fruit showed the disease incidence only level 1.2.

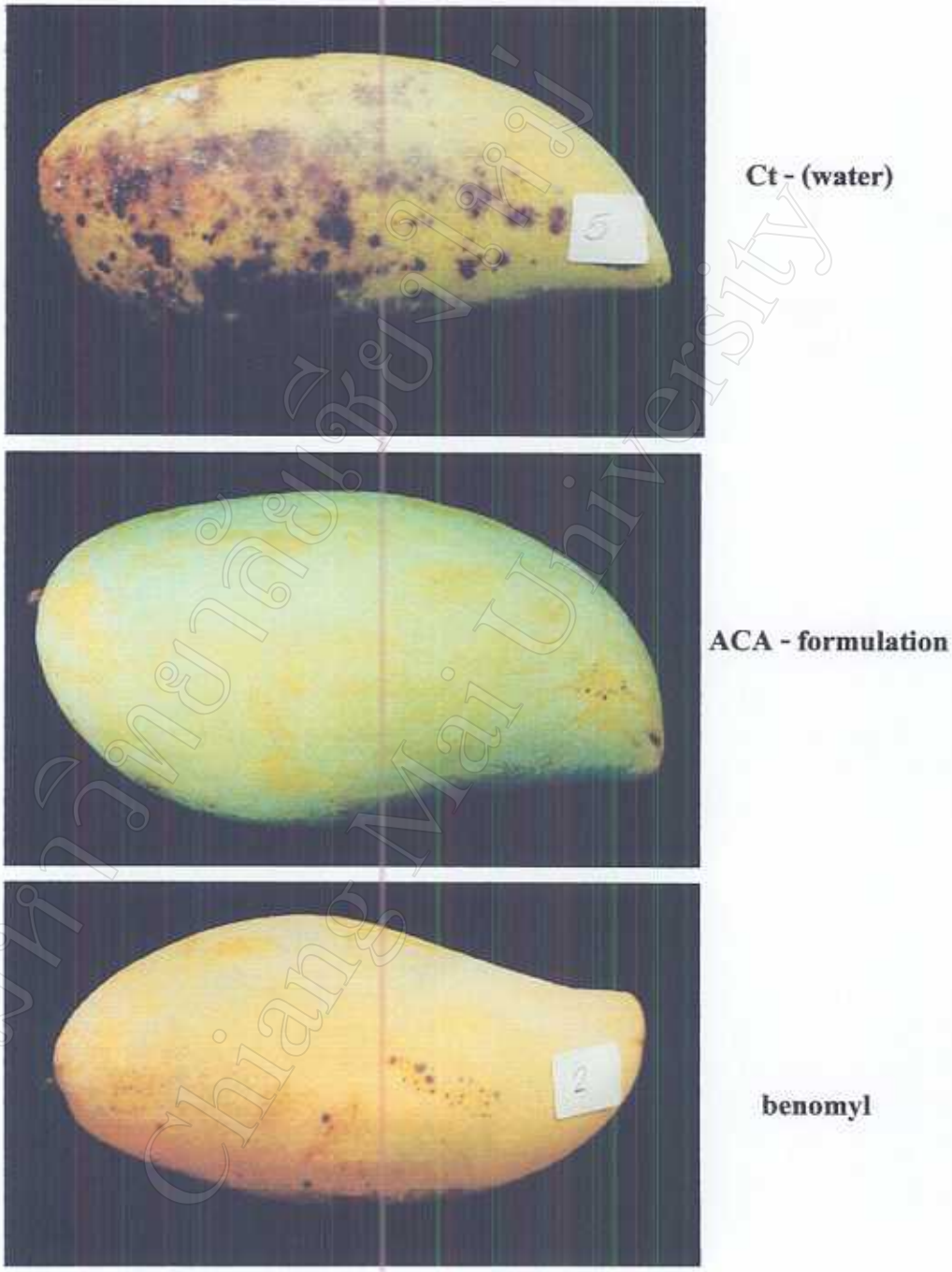

Figure 5.2 Disease incidence on Num-Dok-Mai mango fruit after treated with water (control, 5), 1'acetoxychavical acetate formulation 570 ppm (middle), and benomyl 224 ppm (lower) and kept moist at room temperature for 21 days.

Table 5.3 Efficiency of formulation and benomyl to control postharvest anthracnose disease of mango fruits CV. Nam – Dok – Mai.

Treatments	Details	Disease incidence [1/]
1 Control	Distilled water	5 a [2/]
2 Formulation	570 ppm.	0.65 c
3 Benomyl	224 ppm.	1.2 [b]

CV. 14.73 %

[1/] Mean from 10 replications (fruit), disease rating at 21 days after storage

[2/] Means in the same column followed by significant different of the mean different lsd. $P \leq 0.05$

5.3.4 Conclusion

Sinc active ingredient from galanga, 1′ - acetoxychavical acetate, is attached to essential oil; the formulation of the substance must be produced in the form of emulsifier concentrate (EC.). Successful mixer were Triton X-100 (Primary emulsifier), Agrisol P-135 (secondary emulsifier) and Xylene as solvent in the proportion of 23.8 % active ingredient plus 3.5% Triton – X 100, 3.2% Agrisol P - 135 and 69.5% Xylene, respectively. Minimum fungicidal study confirmed the efficiency of the formulation to control *Colletotrichum gloeosporioides* (Penz.) Sacc. at the concentration of 570 ppm, which showed the disease incident even less than the fruit treated with benomyl.