

Standard Operating Procedures (SOP) for Mixing GM[®] (TC) , (OF) and Mixture Ratio for Field Application



Standard Operating Procedures (SOP) for Mixing Green Muscle[®] Technical Dry Powder (TC) and Oil Flowable Concentrate (OF) formulations and Mixture Ratio for Field Application

1. Introduction:

This Standard Operating Procedures (SOP) for mixing the technical dry powder, formulation, and field application techniques of the Green Muscle[®] formulation would be a useful guidance for locust officers dealing with Green Muscle[®] particularly in the fields.

2. Green Muscle® in the Form of Technical dry Powder (TC):

- This type of formulation produced in the form of dry powder conidia (TC), and formulated in oil on demand.
- It requires special handling during mixing to avoid spillage, dust inhalation, clotting, and sedimentation of the spores.
- Once a packet has been opened, use it immediately, since TC product will decay quickly once exposed to oxygen and moisture.

2.1. Standard Operating Procedure (SOP) for Mixing Green Muscle® TC formulation:

- 2.1.1. Before opening the packet, inject a matching amount of oil into package (e.g. add 1000 ml of diesel oil into a sachet containing 1000 grams of dry TC), using a syringe to avoid spillage and escaping spore dust.
- 2.1.2. Mix the spores thoroughly by hand, through the packet, to form a paste.
- 2.1.3. Cut a corner of the packet and squeeze the contents into a clean container.

- 2.1.4. Rinse the packet out with more diluent (by using another 1000 ml of the diesel oil gradually) to ensure complete transfer of all residual TC into the container.
- 2.1.5. Agitate the container and stir well before use. Try to manually break up any observed clumps and stir the bottom of the container especially well.
- 2.1.6. Sieve the suspension through 150 μ m (100 mesh) or fine nylon-type cloth to avoid any clumped TC, which might block the sprayer nozzles. If the mixing has been done well there should be almost no TC left on the mesh.

Notes:

- a. Two liters of diesel oil are used in this process, 1 liter for mixing and 1 liter for washing the sachet contents.
- b. The final volume of the 2 liters diesel oil mixed with 1 kg spores is more than 2 liters.
- c. The final spore (a.i.) concentration of the prepared suspension is minimum 2.5×10^{13} spores per liter.

2.2. Ratio of mixing the dry technical powder formulation for field application. The tables below show the range of dose rates and areas that can be treated by a 1kg packet.

Table 1: TC dose rate

TC dose rate (a.i. g/hectare):	Spore dose rate (spores/hectare):	1kg TC packet is used to treat:
25g	1.25×10^{12}	40 ha
50g	2.5×10^{12}	20 ha
100g	5×10^{12}	10 ha

Remember that when mixing the oil and TC, the volume changes (in other words: 1kg spores mixed in 2 liters of diesel oil has a volume greater than 2 liters). Always calculate back to the amount of TC.

1. When using motorized mist blower for spraying at a volume application rate (VAR) of 2.5 litre/ha, you can follow the table indicated below

Table 2: VAR: 2.5 L/ha

TC dose rate per hectare	1kg TC packet is used to treat:	Amount of diluent in 1kg paste	Amount of diluent to rinse & add further	Total diluent volume
25g	40 ha	1 Litre	99 Litre	100 Litre
50g	20 ha	1 Litre	49 Litre	50 Litre
100g	10 ha	1 Litre	24 Litre	25 Litre

2. When using spinning disc sprayer for spraying at a VAR of 1 liter/ha:

Table 3: VAR: 1 L/ha

TC dose rate per hectare	1kg TC packet is used to treat:	Amount of diluent in 1kg paste	Amount of diluent to rinse & add further	Total diluent volume
25g	40 ha	1 Litre	39 Litre	40 Litre
50g	20 ha	1 Litre	19 Litre	20 Litre
100g	10 ha	1 Litre	9 Litre	10 Litre

3. When using ULV or aerial sprayers at a VAR of 0.5 liter/ha follow the ratios below:

Table 4: VAR: 0.5 L/ha

TC dose rate per hectare	1kg TC packet is used to treat:	Amount of diluent in 1kg paste	Amount of diluent to rinse & add further	Total diluent volume
25g	40 ha	1 Litre	19 Litre	20 Litre
50g	20 ha	1 Litre	9 Litre	10 Litre
100g	10 ha	1 Litre	4 Litre	5 Litre

3. Green Muscle® in the Form of Oil miscible (OF):

This type of product is produced as an oil miscible flowable concentrate of Green Muscle® spores containing a minimum 2.5 x 10¹³ spores/liter (equivalent to 500g/L). Because it is supplied pre-mixed, it does not cause dust when handling and it mixes relatively easily into diesel. The OF also has a preservative action so that when the drum is opened, the product does not decay quickly.

3.1. Dilution of Green Muscle® OF:

- 3.1.1 This type of formulation usually diluted in appropriate oil such as diesel oil, suitable for use with ULV spraying on demand.
- 3.1.2 Transfer the content of the OF container into a clean bucket.
- 3.1.3 Rinse out the Green Muscle® (OF) container with diluent to ensure complete transfer of contents.
- 3.1.4 Always remember to agitate well before use because the product sediments to the bottom of the container if it has been left in storage for a long time.

3.2. Ratio of diluting the oil miscible flowable concentrate formulation (OF) for field application.

Table 5: (OF) dose rates

OF dose rate (a.i. g/hectare):	Spore dose rate (spores/hectare):	1Liter OF treats:
25g	1.25×10^{12}	20 ha
50g	2.5×10^{12}	10 ha
100g	5×10^{12}	5 ha

1. When using Motorized mistblower for spraying at a volume application rate (VAR) of 2.5 L/ha, follow the ratios indicated below:

Table 6: VAR: 2.5 L/ha

OF dose rate g per hectare	1L OF treats	Amount of diluent to add per 1litre OF	Total diluent volume
25g	20 ha	49 Liters	50 Liters
50g	10 ha	24 Liters	25 Liters
100g	5 ha	11.5 Liters	12.5 Liters

2. When using spinning disc sprayer for spraying at a volume application rate (VAR) of 1 l/ha, you can apply one of the methods indicated below (Table 6):

Table 7: VAR: 1 L/ha

OF dose rate g per hectare	1L OF treats	Amount of diluent to add per 1litre OF	Total diluent volume
25g	20 ha	19 Liters	20 Liters
50g	10 ha	10 Liters	10 Liters
100g	5 ha	5 Liters	5 Liters

3. When using different sprayers, at a volume application rate (VAR) of l/ha, the ratio of mixture is shown on the table below (note: In this table the dose rate is 50 g a.i. per hectare).

Table 8: Ratio of mixture using different sprayers at VAR: 1L/ha

Dilution	ULV ground and Aerial sprayers			Mist-blower
25g GM [®] OF (50g or 100 ml per hectare)	100 ml	100 ml	100 ml	100 ml
Diluents to add	1900 ml	400 ml	900 ml	2400 ml
Total spray volume/ha	2L	0.5L	1L	2.5 L

4. Application Techniques of the Green Muscle®:

- i. The normal application rate of Green Muscle® is 50g per hectare. In some cases, a lower dose rate of 25g per hectare has been used successfully.
- ii. Green Muscle® has a biological action - so there is no immediate visible knock-down, but locusts generally stop feeding within 2 days. Within 6 to 12 days the locusts are dead, but many will have been eaten by natural predators.
- iii. Green Muscle® is compatible with existing pesticide applications techniques.
- iv. It can be applied by air, vehicle mast, boom sprayers (not to be used with an oil formulation) and by Backpack ULV sprayers.
- v. Although Green Muscle® has some residual action, its principle mode of action is through direct contact (this is only true for desert locust in sparse vegetation; in all other cases, one is lucky to achieve 25% direct hit).
- vi. Spray directly at insects as much as possible.

4. Others important factors (indicated below) related to the application techniques of bio-pesticides.

4.1 Checking of the product viability before use:

4.1.1 In order to ensure good result, the product viability should be tested close before field application. A viability of 80% is ideal. For lower viabilities, the dose rate can be adjusted higher. If necessary, contact the supplier, or an approved lab, to assist with viability checking of a small sample.

4.1.2 As a precaution and for better results, the prepared suspension should be used the following day and no longer than three days after preparation.

4.2 Calibration of the spraying equipment and measuring of the flow rate:

Calibration of spray equipment and measuring of flow rate is important in order to apply correct volume application rate and the recommended dose, thus three factors need to be calibrated to achieve an efficient result.

1. Droplet size
2. Emission height
3. Recommended dose

4.2.1 Droplet size:

It is important to have an ideal droplet size. If the droplets are too small they drift away from the target. If the droplets are too large they fall to the ground, and more importantly they waste spores.

Use oil paper to check the droplet size and consistency before spraying.

For the best lethal dose per droplet, the target is:

2,500 ml VAR with a mean droplet size of 95 microns.

4.2.2 Emission height:

It must be set so that bio-pesticides is well distributed over the target area.

Depending on the wind speed, emission height for :

A. handheld sprayers	0.5-2.0 m
B. vehicle mounted sprayers	5-10 m
C. Aircraft	5-10 m

4.2.3 Recommended dose:

To achieve the recommended dose for a given formulation concentration VAR should be regulated using the following formula:

$$\text{Required VAR (L/ha)} = \frac{\text{Recommended dose (g a.i./ha)}}{\text{Formulation concentration (g a.i./L)}}$$

To achieve the required VAR as calculated using above formula, three spraying factors must be set, moreover it is important to understand the relationship between these factors, however if conditions change and one of these factors to be changed, it is important to adjust one or both other factors to apply correct VAR and recommended dose.

1. Track spacing
2. Forward speed
3. Flow rate

4.2.4 Measuring of flow rate for some of spray equipment:

- Collection technique: (e.g. spinning disc sprayer)

Procedure:

- a. Fill the sprayer and position it to deliver the bio-pesticide (diesel oil alone or mixed with Green Muscle[®]) into a measuring cylinder.
- b. Allow the material to flow from the sprayer into the cylinder for specific number of time (usually one minute)
- c. Repeat this process 2-3 time and take the average.

- Loss technique:

This is used when spray liquid cannot be collected easily. (e. g. exhaust nozzle sprayer, air driven pump in aircraft)

Procedure:

- a. Fill the sprayer tank up to a known level.
- b. Spray over the target area using a normal spray technique for specific period of time usually one minute is enough.
- c. Use a measuring cylinder to measure the amount required to refill sprayer tank to it initial level.
- d. The emitted volume is the flow rate per minute
- e. Repeat 2-3 time and take the average.

Calculate the flow rate using the following formula:

$$\text{Flow rate (l/minute)} = \frac{\text{VAR (l/ha)} \times \text{Track Space (m)} \times \text{Speed (Km/hr)}}{600}$$

4.3 Direct spray on the target:

Green Muscle infects locusts by penetrating their cuticle (direct impact by droplets or secondary pickup).

Therefore, it is important to spray direct on the target as much as possible.

4.4 Target status (moving, roosting...etc):

- Efficacy of the treatment is reduced if insects are sheltered by dense crops or are inside bushes, therefore volume and/or dose should be increased.
- Moving insects may leave an area before completion of spraying, so the efficacy of the bio-pesticides may be reduced, therefore treat sufficiently large area to ensure secondary pick-up.
- Spraying early morning or late afternoon, when it is slightly cooler and the UV radiation is not as strong, this will generally yield better results.