*__Original Research Article__*

# Field and in vitro study of Pronos and Dormulin against downy mildew and powdery mildew diseases of grape.


## Abstract

Downy mildewand powdery mildew are destructive diseases of grapes which require application of fungicides. Fungicides are expensive and cause ecological contamination and resistance. Pronos and Dormulin are nutrient mixture which were evaluated against the diseases. The field experiments were conducted against powdery mildew (Sangli, Maharashtra) and downy mildew (Theni, Tamil Nadu) in grapes. *In vitro* experiment against downy mildew conducted at ICAR-National Research Centre for Grapes laboratory. *In vitro* study comprised of 12 treatments. In the case of downy mildew, a field experiment was set with 7 treatments. The foliar application of Dormulin flowering @ 5 g/L of water, and Pronos @ 1.5 g/L of water recorded the lowest percent disease index on leaves, and they were at par with Dormulin (vegetative) followed by 3 sprays of Dormulin (flowering) @ 5 g/L ofwater. In the case of powdery mildew, experiment was set with 10 treatments. On leaves, Pronos (curative) and Pronos (Prophylactic) @ 1.5 g/L of water and Dormulin 1 flowering (curative) @ 5 g/L of water showed the lowest percent disease index on leaves than the untreated control. Therefore,Dormulin flowering @ 5 g/L and Pronos @ 1.5 g/L for downy mildew and Pronos (curative) and Pronos (Prophylactic) @ 1.5 g/L water and Dormulin 1 flowering (curative) @ 5 g/L for powdery mildew were claimed to be effective for controlling diseases. In vitro study showed that Dimethomorph at 1g/L of water was found to be superior over all the treatments with high disease suppression followed by Pronos @1.5gm/L, Dormulin-V1- Flowering grade, and Dormulin-V2- Flowering grade @ 10g/L with slight sporulation. Marketable yield was significantly higher with Pronos (curative) which was on par with Dormulin application as compared to control and fungicide treatment. Use of mineral nutrients in an appropriate proportion is one of the new approaches for plant disease management.



**Keywords**: Bioefficacy, downy, powdery, *In vitro*, fungicide, Pronos and Dormulin.

**Introduction:**

Grape (*Vitis vinifera*) a temperate crop, is known for its commercial and economical attributes. Maharashtra is the leading state in the production of grapes followed by Karnataka, Tamil Nadu and Andhra Pradesh. Grapes a are rich source of vitamins and minerals, along with commendable medicinal qualities that can contribute to a balanced healthy life. Grapes and its products can be considered as potential functional food in reducing hypertension (Sabra et al., 2021). The country has exported 263,075.67 MT of Grapes globally worth of Rs. 2,302.16 crores/ 305.66 USD Millions during the year 2021-22 (Anonymous 2022). Grape crop is affected by various diseases but, mainly by downy mildew (*Plasmoparaviticola*) and powdery mildew (*Erysiphe necator* previously known as *Uncinulanecator*). Downy mildew is the most devastating disease of grapes in the tropical region of the country and mainly appears on the leaves, but also attacks the flower clusters and young fruits. The losses are very high when it attacks the clusters before fruit set as the entire clusters decay and dry. Powdery mildew, is a serious disease during winter season and is seen on all green tender parts. Tocontrolthesediseases, excess fungicides are used which haveraisedserious concerns about food safety, soil and environmental quality. It also enhanced the built up of the fungicide resistance which have dictated the need for alternative disease management techniques (Dordas2008; Kaur 2013). Mineral nutrition plays a very important role in the prevention of plant disease and in the resistance of plants to diseases. The correct management of nutrients in order to control disease in sustainable agriculture had been reported (Huber and Graham 1999).Macro- and microelements had long been recognized as being associated with size, quality and yield of crops, along with changes in levels of the incidence of disease (Rush *et al.*, 1997). Through an understanding of disease interactions with each specific nutrient, the effects on the plant, pathogen and environment can be effectively modified to improve disease control, enhance production efficiency and increase crop quality (Walters and Bingham, 2007). The foliar application of the potassium phosphate fertilizer (1-50-33 NPK) reduced disease incidence on leaves and clusters by 15–65% and severity by 75–90% as compared to untreated vines. These fertilizers were as effective as the fungicide tebuconazole. Foliar sprays of fertilizer mixtures consisting of macro and micronutrients were highly effective, like fungicides, in controlling powdery mildew on both leaves and fruit clusters of grapevines. (Gur *et al*. 2022)

Dormulin, a novel multi-nutrient fertilizer, besides being a yield enhancer is claimed to suppress the pathogens. It has two variants viz. Dormulin (Vegetative) containsmixtures ofnitrogen, phosphorus, potash, calcium, magnesium, silica, and organic carbon while, Dormulin (Reproductive) contains nitrogen in amide form, water-soluble phosphorus, potash, calcium, boron, silica, and organic carbon.Pronos contains organic carbon (3%) and essential nutrients (12%) which possess antifungal properties. In the present study, Dormulin and Pronos were investigated for their bioefficacy against powdery and downy mildew of grapes.

## Material and Methods

**In-vitro study for assessing the efficacy of Dormulin and Pronos against downy mildew.**

**Downy mildew:**

Sensitivity of test of novel fertilizer, Dormulin and Pronos (supplied by Fertis India, Hyderabad) was determined by using a modified 24-well leaf-disc bioassay (FRAC 2003). Healthy leaves were taken from the 6$^{th}$ node from the apex of a growing shoot of the susceptible cultivar, Thompson Seedless and 15-mm disks were cut. The leaf discs were placed upside-down in wells containing 1 ml of 0.5% water agar solution. For the test chemical application, the leaf discs were applied one day prior to inoculation with a spray application machine (10 µl per well), as per the concentrations given in Table 1(a). Each treatment was replicated 6 times. Disc treated with distilled water was kept as control. Leaf discs were inoculated by spraying the suspension onto the leaf discs with 10 uL of a suspension containing 50.000 sporangia/ml of *P. viticola* collected from a single lesion. Plates were incubated at 22°C with alternating periods of 12 hours light and dark. After seven days of incubation, the lesion area was measured using a binocular magnifier. Infected leaf area to percent leaf area of infection, was transformed by considering 100 % infected leaf area in untreated control leaf disc compared to treated disc. The rating of the sporulation of each inoculation point is done using the following scale

0 = no sporulation

1 = light sporulation (difficult to see with naked eye)

2 = sporulation area inferior to the diameter of the deposited inoculum droplet

3 = sporulation area corresponding to the diameter of the deposited inoculum droplet

4 = sporulation area superior to the diameter of the deposited inoculum droplet

Based on the sporulation, at different concentrations percent growth of the pathogen was calculated. The highest concentration at which the isolates is exhibiting sporulation was also noted.

**Field trial to assess the efficacy of Dormulin and Pronos against downy and powdery mildew**

Downy mildew

The experiment was conducted in a vineyards located at Theni, Tamil Nadu. The experiment was laid out in randomized block design with four replications. The variety used was Muscat. Fertis India Limited, Hyderabad, provided the samples of Pronos and Dormulin. Table 1(b). A totalof five sprays including one preventive spray were given whenever the weather conditions were favorable for the development of the disease.

Powdery mildew:

The experiment was conducted in vineyard of Tas-A-Ganesh variety grown on Bower system of training at Tasgaon, Maharashtra. The experiment was laid out in randomized block design with four replications. Two plants per replication per treatment were used for experiment. Table 1(c). Total five sprays including two preventive sprays were given whenever the weather conditions were favorable for development of disease.

Water volume used for spray was calculated based on requirement of 1000 L/ha at full canopy.

Downy mildew incidence on leaves was recorded visually adopting the 0-4 scale, where 0 = nil, 1 = trace to 25, 2 = 26 to 50, 3 = 51 to 75 and 4 = more than 75 leaf area infected (Horsfall and Heuberger 1942). Percent disease index (PDI) was calculated by following formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of numerical ratings} \times 100}{\text{Number of leaves observed} \times \text{Maximum of rating scale}}$$

The ratings on ten leaves were recorded on randomly selected canes. Ten such canes per vine were observed, thus 100 disease observations were recorded per replicate. Four replications for each treatment were considered. Only actively growing downy mildew and powdery lesions were considered for recording ratings. Infection of downy mildew and powdery mildew did not develop on bunches during the period of experimentation.

**Marketable yield**

The yield data was recorded from the downy and powdery mildew trial. The marketable yield from the four replications of each of the treatments and the control was harvested and expressed in Kg grapes/vine and extrapolated to t/ha.

**Statistical analysis**

The PDI data was transformed by using arcsine transformation for leaves and analyzed statistically following Randomized Block Design (RBD) using a Statistical Analysis System (SAS software 9.3). The yield data was analyzed without transformation. Means were compared using Least Significant Difference (LSD) Test.

## Results

***In vitro* study of Pronos and Dormulin against *Plasmoparaviticola*.**

In the in vitro study, all the treatments were found to be significantly superior over untreated control (Table 1). It showed that downy mildew was significantly higher in untreated control than all other treatments. In this experiment it was found that, fungal growth was not observed in standard check fungicide Dimethomorph at 1g/L of water and was found to be superior over all the treatments with a disease suppression of 92.8%. Pronos @1.5g/L of water, Dormulin-V1- Flowering grade and Dormulin-V2- Flowering grade @ 10g/L of water exhibited slight sporulation with a percent disease suppression of 8.3, 85.6, 21.2 respectively as compared to untreated control which had no inhibition of the pathogen **(Fig.1**)

**On field evaluation of downy mildew**

The treatment of Pronos @ 1.5 g/L water and Dormulin flowering @ 5 g/L recorded lowest percent disease index 7.12 and 7.19 respectively which were on par with Dormulin vegetative followed by 3 sprays of Dormulin flowering @ 5 g/L of water (PDI 7.94) during the final observations. Pronos @ 0.75 g/L of water and Dormulin vegetative @ 5 g/L of water recorded a PDI of 10.06 and 11.06 respectively in the last observation. All the treatments were superior over standard check fungicide.

Harvestable yield of grapes in case of Dormulin flowering @ 5 g/L and Pronos @ 1.5 g/L was 14.54 and 13.82 kg/vine respectively which was significantly higher than untreated control (9.09 kg/vine).

**On field evaluation of powdery mildew**

During disease observation, on leaves, Pronos (curative) @ 0.75g/L of water and Pronos (Prophylactic) @ 1.5 g/L of water and Dormulin 1 flowering (curative) @ 5 g/L of water showed lowest percent disease index of 7.5, 7.63 and 8.18 respectively than the untreated control (PDI 25.25) and Myclobutanil 10 % WP (PDI 16.06). Remaining treatments Pronos (Prophylactic) @ 0.75 g/L of water, Pronos (curative) @ 0.75 g/L of water, Dormulin 1 veg (prophylactic @ 5 g/L of water, Dormulin 2 veg (prophylactic) @ 5 g/L of water, Dormulin 2 flowering (curative) @5 g/L of water showed percent disease index 11.13, 11.31, 12.25, 9.81 and 10.6 respectively in last observation.

Harvestable yield of grapes in case of Pronos (curative), Pronos (Prophylactic) @ 1.5 g/L, and Dormulin 1 vegetative (prophylactic) @ 1.5 g/L water was significantly higher i.e. 13.10, 12.77 kg/vine and 12.65 kg/vine respectively than untreated control 5.25 kg/vine. However it was on par with all other fungicide treatments including standard check fungicide myclobutanil 10 % WP (11.41 kg/vine).

**Discussion:**

Downy and powdery mildew are the most devastating diseases of grape which can affect the economic status of the country. Application of fungicide is the most convenient and predominant way for disease control (Peerzada et al., 2020), but the nutrional status of a plant determines histological or morphological structure, properties and the function of tissues to hasten or slow penetration and pathogenesis (Huber et al., 2007).  So, timely application of nutrient helps to control the disease effectively. Use of mineral nutrients in an appropriate proportion is one of the new approaches for plant disease management.

The present study was carried out on the bio-efficacy of novel nutrient mixture Dormulin and Pronos against downy and powdery mildew of grapes. Nutrients are the basic component for disease management as they help to decrease disease severity (Meena et al. 2017) as well as affect plant resistance or susceptibility to disease. Phosphorus (P) and Potassium (K) tend to improve plant health while foliar applications of Boron (B) and Zinc (Zn) help to maintain adequate micronutrient concentrations in vines. Disease resistance is mainly genetically controlled but, has a close association with the nutritional status of the plants or pathogens. The disease suppression is possible through proper nutrient management practices (Huber and Graham 1999). In case of downy mildew, on leaves, the foliar application of Dormulin flowering @ 5 g/L of water recorded lowest percent disease index of 7.19 on leaves than untreated control (15.19). In case of powdery mildew, on leaves, Pronos (curative) and Pronos (Prophylactic) @ 1.5 g/L of water and Dormulin 1 flowering (curative) @ 5 g/L of water showed lowest percent disease index of 7.5, 7.63 and 8.18 respectively as compared to untreated control (25.25). Datnoff et al. (2007) had found that, nutrient played an important role in disease resistance, growth and development of plants. Mineral nutrition may affect plant resistance or susceptibility to disease (Huber 1980). In general, P and K tend to improve plant health, while in most cases, N increased plant susceptibility to disease (Reuveni R et al. 1998). Reuveni et al (1995) studied the exact mode of action of foliar application macro and micronutrients in controlling *E. necator* on grapevines but it was not clearly understood. The macronutrients alone exhibited partial inhibition while macro plus micronutrient mixtures further inhibited conidial germination and caused disruption and shrinkage of hyphae, conidiophores and conidia. Such deformations probably resulted from the osmotic effect of the salts, which disrupted the membrane integrity of fungal cells, causing plasmolysis and leakage of cell content. Reuveni. (1998) further reported nutrient mixtures might also induce local and systemic resistance in grapevines against *E. necator*.Christensen et al.(1980) and Alva et.al (2015) showed that integrating nutrient mixtures especially Boron and Zinc in alternation with fungicides or in mixtures with fungicides in the spray program against grape powdery mildew improved disease control. It was also reported that macro and micronutrient sprays enhanced the production of antifungal and antioxidant metabolites more than fungicide sprays which suggested a possible indirect activity against powdery mildew and the direct inhibiting effect of the nutrients on the pathogen. (Sullivan, (2015); Cabot et.al 2019). Ojha and Jha (2021) noticed that healthy plants will certainly have higher vigour and improved resistance and further proving mineral nutrients had capabilities in disease management. Gur et.al. (2022) reported that, the mixture containing (N, $P_2O_5$, $K_2O$, Zn, B, Mg, Fe, Mn, Cu, Mo, and CO) further reduced disease incidence by 30–90% and disease severity by 85–95% and it was as effective as the fungicide tebuconazole. .

**Tables and Figures.**

**Table 1 (a). Treatments for *in vitro* study of downy mildew of grapes.**

| Sr. no. | Treatment details | Dose (g/L) |
|---|---|---|
| 1 | Pronos | 0.75 |
| 2 | Pronos | 1.5 |
| 3 | Dormulin V1-Vegetative grade | 5.0 |
| 4 | Dormulin V2-Vegetative grade | 5.0 |
| 5 | Dormulin V1-Flowering grade | 5.0 |
| 6 | Dormulin V2-Flowering grade | 5.0 |
| 7 | Dormulin V1-Vegetative grade | 10 |
| 8 | Dormulin V2-Vegetative grade | 10 |
| 9 | Dormulin V1-Flowering grade | 10 |
| 10 | Dormulin V2-Flowering grade | 10 |
| 11 | Dimethomorph | 1 |
| 12 | Untreated Control | 0 |

**Table 1 (b). Treatments for field trial of downy mildew**

| Sr. no. | Treatment Details | Dose (g or ml)/L |
|---|---|---|
| 1. | Pronos | 0.75 |
| 2. | Pronos | 1.5 |
| 3. | Dormulin vegetative | 5.0 |
| 4. | Dormulin flowering | 5.0 |
| 5. | Dormulin vegetative followed by 3 sprays of Dormulin flowering | 5.0 |

| | | |
|---|---|---|
| 6. | Cymoxanil 8% +Mancozeb 64% | 2.0 |
| 7. | Untreated Control | - |

**Table 1 (c). Treatments for field trial of powdery mildew**

| Sr. no. | Treatment details | Dose (g or ml)/L |
|---|---|---|
| 1. | Pronos (Prophylactic) | 0.75 |
| 2. | Pronos (curative) | 0.75 |
| 3. | Pronos (Prophylactic) | 1.5 |
| 4. | Pronos (curative) | 1.5 |
| 5. | Dormulin 1 vegetative (prophylactic) | 5.0 |
| 6. | Dormulin 1 flowering (curative) | 5.0 |
| 7. | Dormulin 2 vegetative (prophylactic) | 5.0 |
| 8. | Dormulin 2 flowering (curative) | 5.0 |
| 9. | Myclobutanil 10 % WP | 0.4 |
| 10. | Untreated Control | 0 |

**Table 2. Details of treatments for *in vitro* study of downy mildew.**

| Tr. No. | Treatment detail | Dose (ml or g/ Liter) | Percent Disease suppression |
|---|---|---|---|
| **T1** | Pronos | 0.75 | 8.3 |
| **T2** | Pronos | 1.5 | 85.6 |
| **T3** | Dormulin V1 Vegetative grade | 5.0 | 21.2 |
| **T4** | Dormulin V2 Vegetative grade | 5.0 | 14.1 |
| **T5** | Dormulin V1 flowering grade | 5.0 | 65.2 |
| **T6** | Dormulin V2 flowering grade | 5.0 | 59.3 |
| **T7** | Dormulin V1 Vegetative grade | 10 | 37.2 |
| **T8** | Dormulin V2 Vegetative grade | 10 | 30.2 |
| **T9** | Dormulin V1 flowering grade | 10 | 78.1 |
| **T10** | Dormulin V2 flowering grade | 10 | 70.3 |
| **T11** | Dimethomorph | 1 | 92.8 |
| **T12** | Untreated Control | - | - |

**Table 3. Bio-efficacy ofDormulin and Pronos in management of downy mildew on leaves after fruit pruning on grapes.**

| Tr No. | Treatment Details | Dose (g/ml/L ) | 1st obs | 2ndObs | 3rdObs | 4thObs | PDC | Yield/vine (kg) | Tonnes/ ha | Percent increase in yield |
|---|---|---|---|---|---|---|---|---|---|---|
| T1 | Pronos | 0.75 | 0.00 (0.00) | 1.43 (1.56) | 1.87 (3.25) | 3.24 (10.06) | 17.97 | 11.09 | 20.05 | 22.03 |
| T2 | Pronos | 1.5 | 0.00 (0.00) | 1.08 (0.69) | 1.50 (1.75) | 2.75 (7.12) | 30.37 | 14.54 | 26.28 | 59.95 |
| T3 | Dormulin vegetative | 5.0 | 0.00 (0.00) | 1.51 (1.81) | 1.85 (2.50) | 3.40 (11.06) | 13.92 | 11.77 | 21.28 | 29.51 |
| T4 | Dormulin flowering | 5.0 | 0.00 (0.00) | 1.11 (0.75) | 1.54 (1.75) | 2.77 (7.19) | 29.87 | 13.82 | 24.98 | 52.04 |
| T5 | Dormulin vegetative followed by 3 sprays of Dormulin flowering | 5.0 | 0.00 (0.00) | 1.19 (0.94) | 1.63 (2.25) | 2.90 (7.94) | 26.58 | 12.78 | 23.10 | 40.60 |
| T6 | Cymoxanil 8% +Mancozeb 64% | 2.0 | 0.00 (0.00) | 2.34 (5.00) | 2.74 (6.50) | 3.67 (13.00) | 7.08 | 12.09 | 21.85 | 32.990 |
| T7 | Untreated Control | - | 3.94 (2.26) | 2.69 (6.75) | 3.08 (9.25) | 3.95 (15.19) | - | 9.09 | 16.43 | - |
| **CD ($p$ = 0.05)** | | | **0.18** | **0.17** | **0.16** | **0.25** | **-** | **3.87** | | |

*= Figures in parenthesis indicate arcsine transformed averages

**Table 4. Bio-efficacy of Dormulin and Pronos in control of powdery mildew on leaves after fruit pruning on grapes.**

| Tr. no. | Treatment details | Dose/L a.i. (g) | PDI of powdery mildew (%) on leaves | | | | PDC | Yield/ vine (kg) | Tonnes / ha |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1st obs | 2nd Obs | 3rd Obs | 4th Obs | | | |
| 1. | Pronos (Prophylactic) | 0.75 | 0.00 (0.00) | 2.75 (7.06) | 3.22 (9.94) | 3.40 (11.13) | 32.80 | 10.33 | 18.67 |
| 2. | Pronos (curative) | 0.75 | 0.00 (0.00) | 2.73 (7.00) | 3.20 (9.81) | 3.43 (11.31) | 32.21 | 10.41 | 18.82 |
| 3. | Pronos (Prophylactic) | 1.5 | 0.00 (0.00) | 2.06 (3.75) | 2.40 (5.44) | 2.84 (7.63) | 43.87 | 12.77 | 23.08 |
| 4. | Pronos (curative) | 1.5 | 0.00 (0.00) | 2.00 (3.56) | 2.34 (5.13) | 2.82 (7.5) | 44.26 | 13.10 | 23.68 |
| 5. | Dormulin 1 veg (prophylactic) | 5.0 | 0.00 (0.00) | 2.74 (7.06) | 3.17 (9.63) | 3.56 (12.25) | 29.64 | 11.87 | 21.46 |
| 6. | Dormulin 1 flowering (curative) | 5.0 | 0.00 (0.00) | 2.12 (4.00) | 2.61 (6.38) | 2.94 (8.18) | 41.89 | 12.65 | 22.87 |
| 7. | Dormulin 2 veg (prophylactic) | 5.0 | 0.00 (0.00) | 2.44 (5.44) | 2.76 (7.19) | 3.20 (9.81) | 36.75 | 11.54 | 20.86 |
| 8. | Dormulin 2 flowering (curative) | 5.0 | 0.00 (0.00) | 2.45 (5.5) | 2.82 (7.56) | 3.24 (10.06) | 35.96 | 11.72 | 21.18 |
| 9. | Myclobutanil 10 % WP | 0.4 | 0.00 (0.00) | 3.47 (11.6) | 3.74 (13.5) | 4.06 (16.06) | 19.76 | 11.41 | 20.62 |
| 10. | Untreated Control | 0 | 3.88 (11.33) | 4.60 (20.8) | 4.79 (22.8) | 5.06 (25.25) | - | 5.25 | 9.49 |
| **CD ($p$ = 0.05)** | | | **0.25** | **0.25** | **0.27** | **0.44** | | **2.83** | |

*= Figures in parenthesis indicate arcsine transformed averages

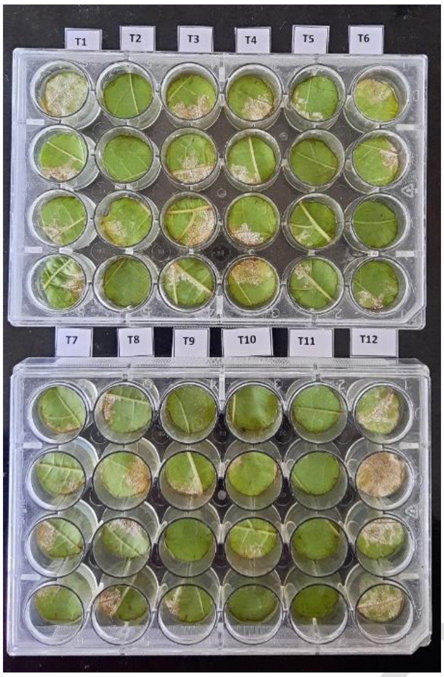


**Fig. 1 In vitro study of Pronos and Dormulin against *Plasmoparaviticola***

**Conclusion**

Dormulin proved to be an effective fertilizer to control both powdery and downy mildew. Probably, the dual action of the chemicals in the lysis of fungal cells as well as disruption of the fungal DNA rendered it effective against grapevine diseases. Pronos and Dormulin together gave a synergistic effect in managing the disease which brings forward the fact that mineral nutrient fertilizer could be the key to successful disease management and form an integral part of integrated disease management of grapes.

**Abbrevations:**

| **Sr. No** | **Abbrevations** | |
|---|---|---|
| **1** | **%** | **Per Cent** |
| **2** | **°C** | **Degree Celsius** |
| **3** | **a.i** | **Active ingredient** |
| **4** | **FRAC** | **Fungicide Resistance Action Committee** |

| | | |
|---|---|---|
| **5** | **g** | **Gram** |
| **6** | **ha** | **Hectare** |
| **7** | **kg** | **Kilogram** |
| **8** | **L** | **Litre** |
| **9** | **MT** | **Metric tonnes** |
| **10** | **PDC** | **Per cent disease control** |
| **11** | **PDI** | **Percent Disease Index** |
| **12** | **USD** | **United States Dollar** |
| **13** | **WP** | **Wettable powder** |

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