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Biostimulants an important nonchemical alternative to pesticides for management of virus disease in Muskmelon

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Abstract

Background: Use of chemical pesticides for controlling viral disease in different crops leads to the development of various problems, viz. residue in sprayed crop, pest resistance, pest resurgence and environmental pollution. It was necessary to find some products that were environmentally safe. So, during this study, biostimulants, viz. buttermilk, kaolin, vegetable oil, neem oil, horticultural mineral oil and lemon grass oil, were tested at different concentrations for the management of viral diseases in muskmelon.

Results: Among these biostimulants, spray of buttermilk at 20% concentration was found to be highly effective with minimum % disease incidence (50.8%) and % disease severity (21.3%), followed by kaolin 2%. Other treatments were also found to be significantly effective in the management of viral disease than the control. In addition to disease control, buttermilk at 20% was also found to increase number of fruits per plant (2.2), number of marketable fruits per plant (2.2), fruit weight (983.3 g), chlorophyll content (26.91 mg m^{-2}) and photosynthetic activity (43.80 mg m^{-2}). Similar to buttermilk 20%, kaolin 2% has also been observed to have significant effect on all abovementioned traits.

Conclusion: As buttermilk 20% spray was found to increase growth and yield of sprayed plant along with controlling viral disease, it can act as a good alternative for the pesticide spray in future because spray of pesticide majority time leads to residue problem, pest resistance and pest resurgence problem.

Keywords: Buttermilk, Kaolin, Lemon grass oil, Melons, Neem oil, Physiology, Viral disease

Background

Melon (Cucumis melo L.) is a fresh vegetable and dessert fruit that can also be cooked, dried or processed for juice and flavoring. Melon seeds can be roasted and eaten like nuts which are sources of high-quality cooking oil along with protein-rich seed flour (McCreight et al. 2011). Like other cucurbits, melons are infected with various viruses around the world. They are susceptible to various viral diseases and are attacked by more than 30 viruses (Zitter et al. 1996), including *Cucumber mosaic virus* and *Zucchini yellow mosaic virus*, which cause serious damage to muskmelons (Sharma et al. 2012) Incidence of other viruses, viz. *Melon Necrotic Spot Virus*, *Muskmelon yellows virus* (MYV) and CGMMV in muskmelon, has also been reported from various parts of world including India (Yin et al. 2014). Besides abovementioned viruses, different DNA viruses viz. *Tomato leaf curl New Delhi virus* and *Tomato leaf curl Palampur virus*, have also been found to infect the muskmelon in Punjab and other parts of world (Dhkal et al. 2020).

Many plants produce volatile essential oils that are thought to serve as defenses against pathogens and insectivores. These naturally occurring substances are known to have a wide range of biological activities, including toxicity and repellency against certain insect pests (Isman 2000). Many workers also found the effectiveness

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of natural products, viz. milk, whey, kaolin, natural oils (Grazo et al. 2003). However, the widespread commercialization of essential plant oils and natural products as crop protection products has lagged behind, often because crucial data on their efficacy and optimal application methods are lacking (Isman 1997). This study was designed to evaluate the effectiveness of different biorationals for the treatment of muskmelon viral diseases, as there are no reports of the effectiveness of biorationals in the treatment of muskmelon viral diseases.

Methods

Crop establishment and transplanting

A nursery of muskmelon was raised in (15 × 10 cm, 100) gauge thick polyethylene bags filled at the base with equal proportions of well-rotted manure and soil. Two seeds per bag of the Punjab Sunehri muskmelon variety were sown to a depth of 1.5 cm in the first half of February. Seedlings were maintained as mentioned in the Package of Practices for Growing Vegetables, PAU, Ludhiana (Anonymous 2017). One-month-old two-leaf stage seedlings were then transplanted in randomized block design on either side of 3-m-wide beds at a vegetable research farm, Department of Vegetable Sciences, in the first two halves of March 2017 and 2018.

Spray application

One day post-transplantation, crop plants were sprayed with oils, viz.. vegetable oil, neem oil, horticultural mineral oil, lemon grass oil, buttermilk and anti-transpiring kaolin. These solutes were procured from different sources and dissolved in water with the help of emulsifier, viz. tween 20 from Agdia Inc (Elkhart, USA), to get desired concentrations (Table 1). The unsprayed plants and mock sprayed plants (i.e., water) served as control. All the sprays of different treatments were applied up to 49 days (7 weeks) at weekly interval. Five plants per replication for each treatment were used with total 3 replications in randomized block design.

Occurrence of viruses in different spray treatments

Antisera of some important viruses, viz.. CMV-I, CMV-II and ZYMV procured from Agdia Inc (Elkhart, USA), were used for serological detection of these viruses in leaf samples collected from different treatments. The DAS/TAS ELISA was performed as per manufacturer's protocol. For detection of whitefly transmitted begomovirus, PALIv722 and PARIc 1960 primer pairs by Chatchawankanphanich and Maxwell (2002) were used in polymerase chain reaction. Total DNA from 100 mg leaf tissue of symptomatic and nonsymptomatic muskmelon was extracted using Lodhi et al (1994) protocol for PCR. PCR was performed in a 10 µl reaction volume containing 2 µl of DNA, 0.6 µl of primer pairs PALIc and PALIv (Chatchawankanphanich and Maxwell (2002) 20 pmol/ml each, 3.5 µl Master Mix (EmeralAmp®GT PCR Master Mix) and 3.3 µl nuclease free water for each sample. PCR was carried out under following conditions: 94°C for 3, 35 cycles of 50 s at 94°C, 45 s at 52°C, and 90 s at 72°C with a final elongation of 15 min at 72°C. The PCR products were analyzed on 1% agarose gel with 1 Kb DNA marker (G-Bioscience, USA).

Effect of spray treatments on pathological parameters

Among various pathological parameters, % disease incidence and % disease severity were important parameters of muskmelon crop. For measuring disease, severity scale, given by Dhkal (2018), was used. These both were measured by using following formula:

$$\%\text{disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Disease severity index (DI) = $\{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4)\ \text{nt}\ (\text{nc} - 1)\}$ 100, where, n_0, n_1, n_2, n_3 and n_4 are the number of plants with score 0, 1, 2, 3 and 4, respectively, nt is the total number of plants and nc is the total number of categories (5).

Effect of spray treatments on growth parameters

Vine length and number of primary branches 2 important growth parameters were measured in this study. Vine

Table 1 Solutes used for the spray at different concentration

Treatments	Source	Concentrations used
Vegetable oil	Jivo Wellness Pvt Ltd, Canada	0.5, 1 and 2%
Neem oil	Achook, Godrej Agrovet Ltd	0.5, 1 and 2%
Horticultural mineral oil	MAK- HMO, MAK Lubricants, Bharat Petroleum, India	0.5,1 and 2%
Lemon grass oil	Marayoor Forests, Forest Department, Kerala	0.1, 0.2 and 0.5%
Anti-transpirant Kaolin	HiMedia Laboratories Pvt. Ltd., India	0.5, 1 and 2%
Butter milk	Verka, Milk Producer's Union. Ltd., India	5, 10 and 20%

length of all plants from each treatment was measured in cm with the help of measurement scale, and numbers of primary branches were counted manually.

Effect of spray treatments on yield parameters

Among yield parameters, total number of fruits per plant, total number of marketable fruits per plant and average fruit weight were measured during this study. Total number of fruits and number of marketable fruits per plant were visually counted, whereas average fruit weight was calculated by measuring the total weight of marketable fruits/total number of marketable fruits.

Effect of spray treatments on physiological parameters

Effect of sprays on important physiological parameters, viz. chlorophyll content, photosynthetic activity, stomatal conductance and transpiration, was measured. These parameters were recorded at the time of harvesting.

Chlorophyll content

Chlorophyll content was measured by using chlorophyll concentration meter (Apogee Instruments Inc, Logan, USA). For measuring chlorophyll, fourth leaf from the top of plant was selected. The leaf was placed in between the sensors of chlorophyll concentration meter, and chlorophyll content (mg/m^2) was measured.

Measurement of photosynthetic activity, stomatal conductance and transpiration

Photosynthetic activity (μmols m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}) and transpiration (mmol m^{-2} s^{-1}) were measured by selecting fourth leaf from the top of plant. To measure these parameters, infrared gas analyzer (IRGA) (ADC LCi, Analytical Development Company Ltd, UK) was used.

Data analysis

Statistical analysis was done by applying analysis of variance in software Windostat Version 9.3

Results

Occurrence of viruses in different spray treatments

Oils, buttermilk and kaolin emerged as an important component of integrated pest management program. These components either protect crop plants directly from the infection of virus by acting on the virus or they protect plants indirectly by affecting the behavior of the vector of viral pathogens that ultimately affects the viral transmission. These products also reported to improve the plant physiology that helps the plant to cover up the pathogen infection. ZYMV, CMV-I and CMV-II were detected as a viral pathogens infecting muskmelon crop during 2017 and 2018 with the help of ELISA (Table 2).

Table 2 Serological and molecular detection of the viruses present in different spray treatments

S. no	Treatments	CMV-I	CMV-II	ZYMV	Begomovirus
1	Vegetable oil 0.5%	+	−	+	+
2	Vegetable oil 1%	+	−	−	+
3	Vegetable oil 2%	−	−	−	+
4	Neem oil 0.5%	−	+	+	+
5	Neem oil 1%	+	−	−	+
6	Neem oil 2%	−	−	−	+
7	Horticulture Mineral Oil 0.5%	−	−	−	+
8	Horticulture Mineral Oil 1%	−	−	−	+
9	Horticulture Mineral Oil 2%	−	−	−	+
10	Lemon Grass Oil 0.1%	+	−	+	+
11	Lemon Grass Oil 0.2%	−	−	−	+
12	Lemon Grass Oil 0.5%	−	−	−	+
13	Kaoline 0.5%	+	−	−	+
14	Kaoline 1%	−	−	−	+
15	Kaoline 2%	−	−	−	+
16	Buttermilk 5%	−	+	−	+
17	Buttermilk 10%	−	−	−	+
18	Buttermilk 20%	−	−	−	+
19	Control + Water	+	+	+	+
20	Control	+	+	+	+

However, primer pairs PALIv722 and PARIc 1960 (Chatchawankanphanich and Maxwell 2002) that is specific for the whitefly transmitted begomoviruses gave an amplification of ~1280 bp (Fig. 1) in all the spray treatments(Table 2). Results of PCR confirmed the presence of begomovirus infection in all spray treatments, whereas no correlation was observed in occurrence of CMV-I, CMV-II and ZYMV and treatments.

Effect of oils, buttermilk and kaolin on pathological parameters

To see the effect of oils, buttermilk and kaolin on pathological parameters, disease incidence and severity of virus symptoms were calculated for all the treatments in both the year and are tabulated in table (3). For interpretation on effectiveness of different treatments, mean disease incidence and severity of both years were worked out that helps to find out the effectiveness of different treatments. Among all the treatments, buttermilk at 20% concentration was found to be highly effective, with percent disease incidence of 50.8%, followed by kaolin 2% (60%). Effect of buttermilk 10% and kaolin 1% on both disease incidence and severity was found to be at par with each other (Table 3). Buttermilk 5%, kaolin 0.5%, vegetable oil 2% and horticulture mineral oil 2% were also

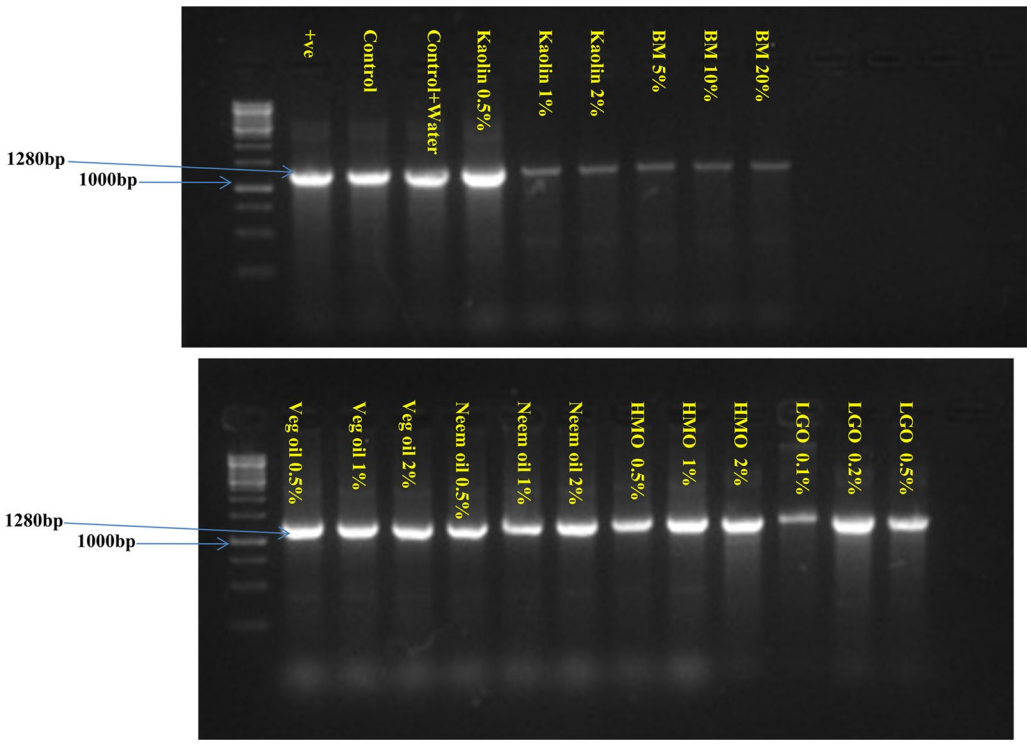

Fig. 1 PCR results in the different spray treatments with PAR1v 722 and PALIc 1960 primer pairs. Lane 1 in both the gels contains 1 Kb DNA ladder (G-Bioscience)

found to be effective in the management of viral diseases in muskmelon. Rest all other treatments did not have any significant effect on disease incidence when compared with controls (Table 3).

Similar to percent disease incidence, % disease severity was also compared between the treatments used. The plants sprayed with buttermilk 20% were found to have least % disease severity (21.3%), whereas the effect of kaolin 2% and buttermilk 10% was at par with 5% disease severity 24.3 and 28%, respectively. The buttermilk 5% and kaolin 0.5% were also found to have significant effect on % disease severity (31.5 and 34.3%) and were at par. All other treatments were also had significant effect on % disease severity than the control (Table 3).Among various treatments that were tested during this study, all buttermilk and kaolin treatments were found to be highly significant in managing viral diseases on muskmelon as these treatments lead to the least % disease incidence and % disease severity.

Effect on growth parameters

Least disease incidence and disease severity on the plants result in the better growth. During this study plant, growth parameters (vine length and primary branches) were also measured. Least virus disease on buttermilk 20% sprayed plant also results in maximum mean vine length (200.3 cm), followed by kaolin 2% (185.1) (Fig. 2). Minimum vine length was observed in the plants sprayed with lemon grass oil 0.1% (95.3 cm), followed by neem oil 0.5% (101.6 cm). Other treatments were also had significant effect on vine length of muskmelon plants (Fig. 2).

These different treatments had also significant effect on the number of primary branches. Maximum primary branches were observed in buttermilk 20% (4.7), followed by buttermilk 10% (4.4). Minimum number of primary branches was in plants sprayed with lemon grass oil 0.1% followed by lemon grass oil 0.1% (Fig. 2).

As observed in case of pathological parameters, similar trend was observed in plant growth parameters also, where buttermilk and kaolin spray was found to be effective in enhancing the growth of the plants. This enhanced growth could be due to the less disease incidence and severity on the sprayed plants.

Effect on the yield parameters

Good growth of plant has positive effect on different yield parameters of the crop plants. This trend was also observed in this experiment where the plants sprayed with buttermilk 20% were found to have least % disease incidence and severity leads to maximum number of

Table 3 Effects of oils, kaolin and buttermilk on different pathological parameters

S.no	Treatment	% Virus disease incidence	% Virus disease severity
1	Vegetable oil 0.5%	100.000^{f}	60.833^{lk}
2	Vegetable oil 1%	100.000^{f}	47.000^{g}
3	Vegetable oil 2%	84.167^{e}	37.000^{ef}
4	Neem oil 0.5%	100.000^{f}	65.667^{lm}
5	Neem oil 1%	100.000^{f}	55.333^{ij}
6	Neem oil 2%	94.167^{f}	46.833^{g}
7	Horticulture Mineral Oil 0.5%	100.000^{f}	58.333^{kj}
8	Horticulture Mineral Oil 1%	100.000^{f}	52.000^{hi}
9	Horticulture Mineral Oil 2%	85.000^{e}	41.000^{f}
10	Lemon Grass Oil 0.1%	100.000^{f}	68.000^{mn}
11	Lemon Grass Oil 0.2%	100.000^{f}	60.000^{jk}
12	Lemon Grass Oil 0.5%	93.333^{f}	47.333^{gh}
13	Kaolin 0.5%	74.667^{d}	34.333^{ed}
14	Kaolin 1%	65.000^{bc}	31.333^{cd}
15	Kaolin 2%	60.000^{b}	24.333^{ab}
16	Buttermilk 5%	71.667^{cd}	31.500^{cd}
17	Buttermilk 10%	64.167^{bc}	28.000^{bc}
18	Buttermilk 20%	50.833^{a}	21.333^{a}
19	Control + Water	100.000^{f}	72.000^{no}
20	Control	100.000^{f}	73.000^{o}
	CD. 5%	7.86	4.84

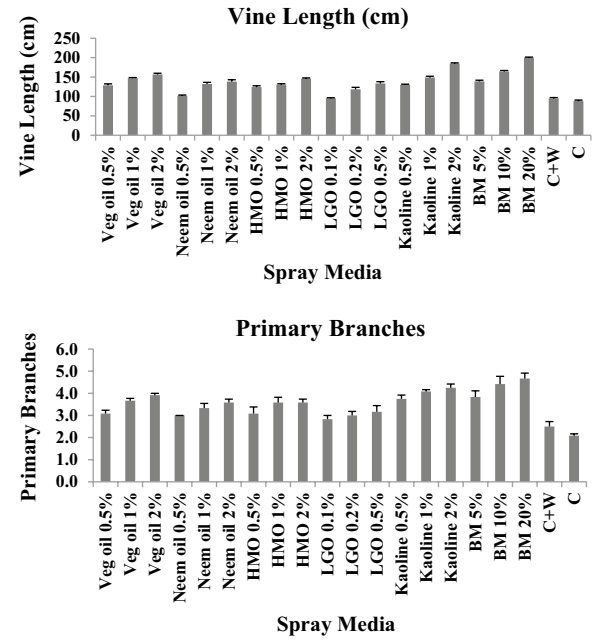

Fig. 2 Effects of oils, buttermilk and kaolin on growth parameters, viz. vine length (cm) and primary branches

fruits (8.4) per plant after 60 days of transplanting, followed by the kaolin 2% (7.2). Minimum number of fruits was observed in the plants sprayed with lemon grass 0.1%, followed by plant sprayed with neem oil 0.5%. All other treatments were also observed to significantly increasing the number of fruits per plant after 60 days of transplanting as compared to control (Fig. 3).

Similar to the number of fruits per plant 60 DAT, number of marketable fruits per plant was also found to be more in buttermilk 20% (2.1), followed by kaolin 2% (1.9) and buttermilk 10% (1.7). Minimum number of marketable fruits was observed in neem oil 0.5%, followed by lemon grass oil 0.1%. Number marketable fruits were significantly more in all other treatments as compared to control (Fig. 3).

Similar to the number of fruits per plant and number of marketable fruits per plant, fruit weight was also found to be higher in case of buttermilk 20% (983.3 g), followed by buttermilk 10% (837.5 g), kaolin 2% (883.3 g) and kaolin

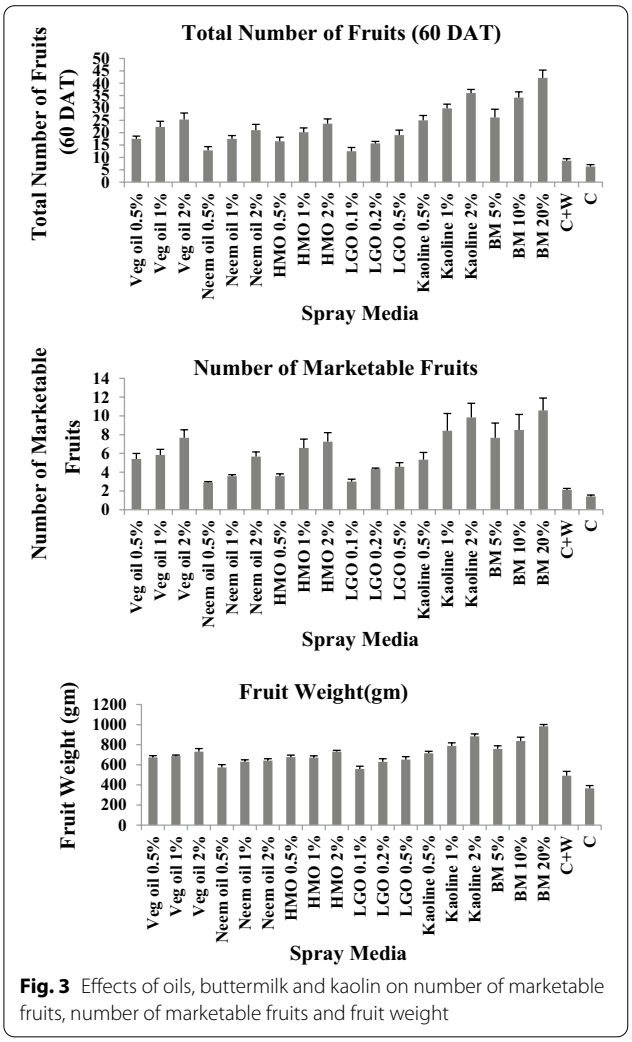

Fig. 3 Effects of oils, buttermilk and kaolin on number of marketable fruits, number of marketable fruits and fruit weight

1% (787.5 g), which was at par with each other, whereas fruit weight in case of buttermilk 5% (758.3 g) and kaolin 0.5% (715 g) was at par with each other. Least fruit weight was observed in lemon grass 0.1% spray (558.3 g), followed by neem oil 1% (574.2 g). All other treatments had significant effect on the mean fruit weight as compared to control (Fig. 3).

Effect of oils, buttermilk and kaolin on the physiological parameters of Muskmelon

Diseases are known to disrupt normal physiological function of a plant. To assess the effect of different sprays on physiological parameters during this study, four physiological parameters, viz. chlorophyll content, photosynthetic activity, stomatal conductance and transpiration of muskmelon plants after spray, were measured. Spray of buttermilk at 20% led to a maximum increase in the chlorophyll content viz. (26.91 mg m^{-2}), followed by the spray of kaolin 2% (18.27 mg m^{-2}), whereas minimum increase in chlorophyll content was observed in spray of horticulture mineral oil at concentrations of 0.5% (2.06 mg m^{-2}) and 1% (3.12 mg m^{-2}). All other treatments also significantly enhanced the chlorophyll content of the sprayed plants than the control (Table 4).

Kaolin spray at 2% and buttermilk spray at 20% had a maximum mean photosynthetic activity of 43.90 µmols m^{-2} s^{-1} and 43.80 µmols m^{-2} s^{-1} that was atpar with each other. Unlike chlorophyll content, minimum photosynthetic activity was observed in plants sprayed with neem oil at 0.5% (23.07 µmols m^{-2} s^{-1}), followed by the plant sprayed with lemon grass oil 0.1% (23.67 µmols m^{-2} s^{-1}). However, other majority of treatments were observed to significantly enhance the photosynthetic activity of sprayed plants, except few treatments (Table 4).

Spray of kaolin 2% was found to enhance the stomatal conductance in the sprayed plants, viz.. 0.24 mol m^{-2} s^{-1}, followed by buttermilk 20% (0.21 mol m^{-2} s^{-1}) (Table 4), whereas plants sprayed with HMO 1% were found to have least stomatal conductance of 0.01 mol m^{-2} s^{-1} that was even less then control plants (Table 4). Majority of spray treatments were found to have significant effect on the stomatal conductance of sprayed plants, except few, viz.. vegetable oil sprays at 0.5% and 1%, neem oil spray at 1 and 2%, HMO spray at 1%, lemon grass spray at 0.1% and buttermilk spray at 5% (Table 4).

Like mean stomatal conductance, mean transpiration was also found to be higher in kaolin 2% sprayed plants (7.59 mmol m^{-2} s^{-1}), followed by buttermilk 20%

Table 4 Effect of oils, buttermilk and kaolin on chlorophyll content, photosynthetic activity, stomatal conductance and transpiration

S. no	Treatment	Chlorophyll content (mg m^{-2})	Photosynthetic activity (µmols m^{-2} s^{-1})	Stomatal Conductance (mol m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})
1	Vegetable oil 0.5%	4.89	24.90	0.07	3.42
2	Vegetable oil 1%	8.50	28.03	0.06	3.15
3	Vegetable oil 2%	10.71	33.37	0.14	5.72
4	Neem oil 0.5%	4.94	23.07	0.19	6.13
5	Neem oil 1%	6.20	39.00	0.02	1.30
6	Neem oil 2%	12.07	31.43	0.06	2.74
7	Horticulture Mineral Oil 0.5%	2.06	26.70	0.12	4.20
8	Horticulture Mineral Oil 1%	3.12	30.90	0.01	0.75
9	Horticulture Mineral Oil 2%	14.33	31.67	0.13	4.18
10	Lemon Grass Oil 0.1%	10.37	23.67	0.07	3.17
11	Lemon Grass Oil 0.2%	14.42	25.83	0.21	6.59
12	Lemon Grass Oil 0.5%	16.94	34.03	0.12	4.75
13	Kaolin 0.5%	13.10	41.07	0.09	4.09
14	Kaolin 1%	15.42	40.13	0.11	5.57
15	Kaolin 2%	18.27	43.90	0.24	7.59
16	Buttermilk 5%	6.47	38.83	0.02	1.31
17	Buttermilk 10%	14.66	42.50	0.11	4.28
18	Buttermilk 20%	26.91	43.80	0.21	6.61
19	Control + Water	3.34	17.80	0.09	4.20
20	Control	1.67	15.63	0.05	2.70
21	General Mean	10.41	31.81	0.11	4.12
	C.D. 5%	2.07	13.12	0.02	0.76

(6.61 mmol m^{-2} s^{-1}) and LGO 0.2% (6.59 mmol m^{-2} s^{-1}) as their activities were found to be at par with each other (Table 4). Minimum mean transpiration was observed in the plants sprayed with HMO 1%, followed by neem oil spray 1% and buttermilk spray 5% (Table 4). Same trend, as observed in case of stomatal conductance, was observed in transpiration also where majority of treatments were found to have significant effect on transpiration (Table 4).

Discussion

The use of pesticides in combating various viral diseases has several limitations, viz. residue problems, resistance in the insect vector of the viral pathogen, and high cost of pesticide spray. Various products found to be effective in this study, viz. buttermilk, kaolin and natural oils, can all be helpful in solving the above problem. Buttermilk was found to be highly effective in the management of viral disease during this study that could be due to whey and whey protein which are the main component of buttermilk. These proteins are known to be effective in management of viral diseases (Abdelbacki et al. 2010). During this study, kaolin was also found to be effective against the viruses as kaolin spray changes the structure, texture and color of plant surface for short period of time and these factors decided the virus transmission efficiency by affecting the response of vector. Similar to present study, Garzo et al. (2003) also found kaolin to be effective in controlling cucumber mosaic virus infection on melons crop by affecting settlement rate and reducing transmission efficiency of aphid. Creamer et al. (2005) observed that the application of kaolin spray significantly reduced the infection of *Beet curly top virus* on chilli. Natural oils were also observed to be effective in the management to viral disease that was similar to the findings of other studies (Rajinimala et al. 2007).

In addition to controlling disease occurrence in muskmelon, buttermilk and kaolin had also acted as a plant growth stimulant, ultimately improving the growth and yield of the sprayed plants. Similarly, Legault (2007) observed that spraying kaolin on cucumber plants significantly increased marketable crop yield. Besides cucurbits, kaolin spray increased the yield of many other crops (Spiers et al. 2004). In contrast to the present results, Russo and Diaz-Perez (2005) observed that application of kaolin clay to peppers did not significantly affect pepper yield. During this study, spray of kaolin was reported to increase the chlorophyll content in the plant that could also be the reason of increased yield of crop in this treatment than the other treatments.

In addition to controlling various viral infections and increasing yield, spraying of natural products had significantly affected various physiological traits of crops. Natural product sprays can affect plant physiology either directly by increasing or decreasing chlorophyll content, photosynthetic activities, stomata conductivity and transpiration of the sprayed plants, or indirectly by protecting the sprayed plants from various biotic or abiotic stresses. It is known that the antiviral response against systemic viral infection was significantly associated with chloroplast function (Gnanasekaran et al. 2019). In this study, buttermilk 20% and kaolin 2% spray treatments were found to increase chlorophyll levels, along with the lowest incidence and severity of the disease. It has been reported that chloroplasts are the main site for the synthesis of salicylic acid and jasmonic acid, which are the main components of the plant defense response. During this study, increase in the chlorophyll levels in buttermilk and kaolin spray treatments could be the reason of vigorous growth that resulted in less viral infection.

Chlorophyll levels are closely related to the rate of photosynthesis as they provide the photosynthetic machinery that allows plants to absorb energy from light and transfer it to chlorophyll a (Porra et al. 1993). With higher chlorophyll content in the leaves, a higher rate of photosynthesis can be sustained (Kura-Hotta et al. 1987). In this study, treatments high in chlorophyll also showed higher photosynthetic activity. Chlorophyll measurements were derived from the same leaves used for measuring photosynthetic activity and were measured at the same phase. For 20% buttermilk, when photosynthesis was high, stomatal conductance and transpiration were comparatively lower than other treatments because much H_2O was used for photosynthesis before the water vapor was released in the transpiration process.

Conclusions

The products like buttermilk, kaolin and different oils were found to be effective and have the potential to become an important component of integrated disease management strategy against viral pathogens. Some of these products are easily available to the farmers and safe to the environment specially buttermilk. In future, the effectiveness of these products can further be increased by using them in combination with each other or by designing their spray schedule with different available insecticide so that they can reduce the pesticide load on crop plants that ultimately solve various important problems like residue of pesticides in food products, pest resistance, pest resurgence and pollution of natural resources and environment that occur due to high use of pesticides. Moreover, during this study spray of buttermilk and kaolin was observed to increase the chlorophyll content of plant. Chlorophyll was earlier reported to be active site for the production of different defense-related products and contain some genes, expression of which

reduces the infection of whitefly transmitted virus. But there is further need to solve some important questions like how these sprays are increasing chlorophyll content, are these sprays responsible for increasing the expression of some defense-related genes present in chlorophyll that provide defense to the sprayed plants.

Abbreviations
HMO: Horticultural mineral oil; et al.: Et alia (co-workers); DAS: Double antibody sandwich; TAS: Triple antibody sandwich; ELISA: Enzyme-linked immunosorbent assay; DAT: Days after transplanting.

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Author contributions
MD conducted the experiment, collected the data, analyzed the data, collected literature and wrote the manuscript. AS supervised and participated in the planning and implementation of the experiments and reviewed the manuscript. SS participated in the planning and implementation of the experiments. All authors read and approved the final manuscript.

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Declarations

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Not Applicable.

Consent for publication
Not Applicable.

Competing interests
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