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# Efficacy of Botanicals against *Suidasia nesbitti* Hughes (Acari: Suidasiidae) in Stored Pearl Millet Grains

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## ABSTRACT

The study was conducted during 2020 at CCS Haryana Agricultural University, Hisar to bioassay the aqueous extract of Aonla fruits, lemon and orange peels against *Suidasia nesbitti* in pearl millet grains. Higher concentrations of botanicals (4 and 8%) caused 100% mortality as no mites were recorded in these treatments after 15 days as compared to significantly higher mites at 0.5 (30 mites), 1 (24.33 mites) and 2% (18 mites) concentration. Among the three botanicals, the orange peel extract was more potent than other two botanicals (anola fruit and lemon peel) as it caused 100% mortality in *S. nesbitti* population. No mite was recorded at all the concentrations after 1day of treatment. Lemon peel at 8% concentration caused 100% mortality in *S. nesbitti* within 3 h of treatment as no mite was recorded. No mite was recovered from 4% lemon peel and 4 and 8% Aonla fruit extract treated grains after 1 day of treatment. Higher concentrations of 4 and 8% of Aonla fruit provided 100% protection against *S. nesbitti* mite mortality. The $LC_{50}$ (concentration at which 50% mortality occurred in *S. nesbitti* population) value was 0.64% in lemon peel extract while it was 1.38% in Aonla fruit extract against *S. nesbitti*. The intercept value was 4.74 and 5.24 for Aonla fruit and lemon peel extract, respectively. Bioassay results revealed acaricidal activity of aqueous extracts of three botanicals against *S. nesbitti*.

**KEYWORDS:** Aonla, mite, orange, pearl millet, grains, acaricidal, lemon peel, *Suidasia nesbitti*

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***Data Availability Statement:*** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## 1. INTRODUCTION

Millet is one of the most important cereals in the world (Slama et al., 2020). The major millet in terms of world production is pearl millet (Shahidi and Chandrasekara, 2013). It is a quick growing, short duration crop (Shashikala et al., 2013) and one of the basic cereals of several Asian and African countries (Martins et al., 2018). Factors like temperature, moisture content, duration of storage and pest infestation affects the stability of grain (Kaur and Dhingra, 2018). Grain provides an abundant source of nutrients to variety of organisms including microorganisms, insects, mites and rodents (Palyvos et al., 2008). Pests infestion in storage are an important problem worldwide (Clemmons and Taylor, 2016). Among the mites, *Suidasia nesbitti* have a short-duration life cycle and rapid reproduction rate. Mites when present in large numbers cause serious damage to stored products and health problems like asthma, acute enteritis (Valbuza et al., 2020) and allergies (Zannou et al., 2013). Control of these mites is done by using chemical methods such as fumigation or spraying with organophosphorous compounds in empty stores (Szlendak et al., 2000). Mangoba and de Guzman Alvindia (2019) reported $LC_{50}$ and $LC_{99}$ values as 0.009 and 0.025 mg $l^{-1}$, respectively against phosphine susceptible strain of S. pontifica whereas, for most resistant strain, the values were 1.501 and 2.407 mg $l^{-1}$, respectively. Disadvantages of synthetic chemical acaricides are resistance development in the pest, phytotoxicity, incompatibility with natural predators, environmental pollution and risks to human health (Rincon, et al., 2019). These problems have highlighted the need to use eco-friendly and safe alternatives for stored mite control. The ecofriendly measures include use of entomopathogenic fungi (Anita, 2017), inert dust (Sabbour et al., 2012) and botanicals (Gulati, 2007) to keep the acarine population below economic threshold level.

Plant products are used for the control of mites associated with post harvest losses (Kumari et al., 2016), against rice stored grain pests (Asangla, 2013) and insect pests of maize (Soujanya et al., 2016). Neem oil, usually used as mite and insect repellent on many crops, contains different bioactive compounds, one of them is azadirachtin (Campos et al., 2016). Higher concentrations of neem based products although effective against plant mites, showed symptoms of phytotoxicity (Venzon et al., 2020). These oils reduce number of eggs, adult emergence and longevity of mites. Exposure period of oil is the most important factor which influences the repellent effect (Klys et al., 2017). Chemical composition of essential oils (EO) varied according to species and variability create difference in their insecticidal activities (Amri et al., 2014). EO have a relatively short efficacy duration period due to their high volatility and quick biodegradability (Tak and Isman, 2017). Radsetoulalova et al. (2020) evaluated the acaricidal effects of EO *viz*., eucalyptol from rosemary, eugenol from clove bud, limonene from citrus fruits and cinnamaldehyde from cinnamon against poultry red mite, *Dermanyssus gallinae* in the concentrations ranging from 0.002 to 0.06 µl $cm^{-2}$. The highest mortality was observed with eugenol. The miticidal and repellent activity of plant EO against *Tetranychus urticae* were actively explored by Benelli et al., 2018, Ebadollahi et al., 2016, Mar et al., 2018. Plant extract of neem (Hossain et al., 2013) and citrus plants (Cabedo et al., 2021) evaluated against *Polyphagotarsonemus latus*. In orange peels, d-Limonene component acted as a repellent against pests (Klimek-Szczykutowicz et al., 2020). Aboelhadid et al. (2016) evaluated the effect of EO of lemon on parasitic mite, *Sarcoptes scabiei* infection in rabbits. In view of above facts, extracts of Anola fruits, lemon and orange peels were evaluated against *S. nesbitti* in stored pearl millet grains during present study.

## 2. MATERIALS AND METHODS

The study was conducted during 2020 at CCS Haryana Agricultural University, Hisar. Anola fruits, lemon and orange peels were collected from sector-15 market (Hisar) and *Suidasia nesbitti* from the department of zoology of CCSHAU Hisar. Anola fruits, peels of lemon and orange (100 g each) were crushed and soaked in 100 ml of distilled water separately for 48 h with intermittent shaking. After 48 h, the solutions were filtered through the muslin cloth and collected in glass bottles. The filtrates were considered as crude aqueous extracts and below mentioned dilutions were prepared from these stock solutions using distilled water by volume to volume dilution method.

Table 1: Botanicals used against *Suidasia nesbitti*

| Sl. No. | Botanical extract used | Concentrations evaluated |
|---|---|---|
| 1. | Aonla (*Phyllanthusemblica*) fruit | 0, 0.5, 1, 2, 4, 8 |
| 2 | Orange (*Citrus sinensis*) peel | 0, 0.5, 1, 2, 4, 8 |
| 3. | Lemon (*Citrus limon*) peel | 0, 0.5, 1, 2, 4, 8 |

Experiments were conducted separately for each botanical with different treatments. To evaluate the efficacy of aqueous extract of Aonla fruits against *S. nesbitti*, pearl millet grains were treated with five dosages viz., 0.5, 1, 2, 4, 8% in separate sets through uniform mixing. Each set was replicated three times. In each replicate, 10 *S. nesbitti* pairs/5 g grains were released. Observations on the number of live mites under stereo zoom microscope were counted in each replicate after 15 m, 30 m, 1 h, 2 h, 3 h, 1 day, 2 days, 7 days and 15 days of treatment. In identical experiments, the effects of aqueous extracts of lemon and orange peel

under above mentioned five dosages were evaluated after 15 m, 30 m, 1 h, 2 h, 3 h, 1 day, 2 days, 7 days and 15 days of treatment.

Non-infested pearl millet grains were kept as control under triplicate conditions. Immobile mites were probed with bird feather's pick, if they failed to respond with leg movement, they were considered as dead. The observed mortality was converted into per cent mortality and corrected mortality was obtained after deducting the mortality in control treatments. The observed data was used for the calculation of $LC_{50}$.

### *2.1. Statistical analysis*

Critical difference (CD) was calculated to know the efficacy of the different treatments in reducing the *S. nesbitti* population in pearl millet grains. Log concentrations, probability regression including a control mortality correction as an offset for natural mortality were estimated using Probit analysis. The $LC_{50}$ i.e. lethal concentration required for 50% mortality of the adult stage of *S. nesbitti* was calculated.

## 3. RESULTS AND DISCUSSION

Aqueous extracts of Aonla(*Phyllanthus emblica*)fruits, lemon (*Citrus sinensis*) and orange (*Citrus limon*) peels were evaluated under *in vitro* conditions to determine their acaricidal activity against *S. nesbitti* in pearl millet grains at 27±1°C, 80–85% relative humidity.

### *3.1. Acaricidal activity of aonla fruit extract*

The data pertained to acaricidal activity of aqueous extract of Aonla fruit against *S. nesbitti* population on pearl millet grains is presented in Table 2. The untreated grains harboured significantly more number of *S. nesbitti*/ 5 g grains (22.5 mites) compared to treated grains. Higher concentrations were more effective in reducing the number of live mites as compared to lower concentrations.

With an initial inoculum of 10 mite pairs, significantly less number of mites were recorded at 8 (4.67 mites), 4% (5.20 mites) concentrations; both being comparable with each other; than at 2 (9.73 mites), 1 (12.53 mites) and 0.5% (16.57 mites) concentration and control (CD=0.620; $p$=0.05).Duration of the treatment showed significant reduction in *S. nesbitti* population. From the initial 20 mites, it significantly decreased the *S. nesbitti* numberto 15.84, 12.83, 10.56, 9.28, 8.33, 6.56 and 6.17 mites after 15 m, 30 m, 1 h, 2 h 3 h, 1 day and 2 days of the treatment (CD=0.877; $p$=0.05). Irrespective of the treatment, number of mites recovered after 7 and 15 days of treatment was 10.72 and 18.39 mites. The number of mites recorded after 1 h and 7 days were statistically at par with each other. Similarly, non-significant difference in number of mites was observed after 1 and 2 day of treatment. Statistically significant interaction between the treatments and observation periods was recorded (CD=1.960; $p$=0.05) which indicated that all the treatments were significantly better than control treatment. At 8 and 4% concentrations, no live mites were recorded after one day of the treatment. Concentration dependent activity against *S. nesbitti* (Rani, 2000) and *T. putrescentiae* (Gulati, 2007a,b, Anita et al., 2014) was earlier recorded by many workers. Rani (2000) also reported higher concentration (0.75 ml $kg^{-1}$) of Aonla, soyabean, coconut, karadi and safflower oils more effective against *S. nesbitti* than lower concentrations. Saponins are bioactive compounds in Aonla fruits which have insecticidal and cytotoxic properties against certain insects. The saponins show antifeeding activity against mite, *Oligonychus*

Table 2: Effect of Aonla fruit extract against *Suidasia nesbitti* in pearl millet grains

| Treatments (%) | Number of mites after treatment | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 m | 15 m | 30 m | 1 h | 2 h | 3 h | 1 day | 2 day | 7 day | 15 day | |
| 0.50 | 20.00 | 18.67 | 17.33 | 16.00 | 15.00 | 4.00 | 10.67 | 8.33 | 15.67 | 30.00 | 16.57 |
| 1.00 | 20.00 | 16.33 | 13.67 | 10.33 | 8.33 | 8.33 | 5.33 | 3.33 | 15.33 | 24.33 | 12.53 |
| 2.00 | 20.00 | 14.67 | 10.33 | 8.00 | 6.33 | 5.00 | 3.33 | 3.33 | 8.33 | 18.00 | 9.73 |
| 4.00 | 20.00 | 13.67 | 8.67 | 5.00 | 3.33 | 1.33 | 0.00 | 0.00 | 0.00 | 0.00 | 5.20a |
| 8.00 | 20.00 | 11.67 | 7.00 | 4.00 | 2.67 | 1.33 | 0.00 | 0.00 | 0.00 | 0.00 | 4.67a |
| Control (Untreated grains) | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 22.00 | 25.00 | 38.00 | 22.50 |

CD ($p$=0.05) for Treatment=0.620; SE (m)=0.221; CD ($p$=0.05) for Observation period=0.877; SEm±=0.312; CD ($p$=0.05) for Treatment×Observation period=1.960; SEm±=0.698; Values with the same superscript in a row/column do not differ significantly

*indicus* and two caterpillar species, *Hyphantria cunea* and *Malacosoma americanum* (Chaieb, 2010).

### 3.2. Acaricidal activity of lemon peel extract

The results on the acaricidal activity of aqueous extract of lemon peel extract against *S. nesbitti* are presented in Table 3 on pearl millet grains at different investigating times.

Mites responded to lemon peel extract in a concentration dependent manner i.e. lower number of live mites was observed with higher concentrations. Out of an initial number of 10 mite pairs, significantly less number of mites 9.63, 8.87, 8.57, 4.57, 3.87 were recovered from 0.5, 1, 2, 4 and 8% concentration as compared to 22.5 mites in untreated grains (CD=0.382; $p$=0.05). The number of live mites at 1 and 2% concentration showed non-significant difference with each other. Duration wise, *S. nesbitti* counts remained significantly low after 15 min (13.94 mites), 30 min (9.84 mites), 1 h (8.39 mites), 2 h (6.34 mites), 3 h (5.34 mites), 1 day (4.39 mites) and 2 day (4.61 mites) of post treatment (CD=0.540; $p$=0.05). Thereafter, the number of mites increased to 9.22 and 14.61 after 7 day and 15 days after treatment although the counts remained significantly low than the initial number (Table 3). Significant interaction was observed between the concentrations and observation periods (CD=1.207; $p$=0.05) (Table 3) which indicated that higher concentration was significantly better than lowerconcentrations at all durations. Lemon peel at 8% concentration caused 100% mortality in *S. nesbitti* within 3 h of treatment as no mite was recorded. No mite was recovered from 4% lemon peel treated grains after 1dayof treatment. Aboelhadid et al. (2016) evaluated the effect of essential oil of *C. limon* on parasitic mite, *Sarcoptes scabiei* infection in rabbits. *In vitro* application at 10 and 20% concentration on infected parts caused 100% mortality in mites within 24 h.

Table 3: Effect of lemon peel extract against *Suidasia nesbitti* in pearl millet grains

| Treatments (%) | Number of mites after treatment | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 m | 15 m | 30 m | 1 h | 2 h | 3 h | 1 day | 2 day | 7 day | 15 day | |
| 0.50 | 20.00 | 15.33 | 11.00 | 9.33 | 4.67 | 3.00 | 2.33 | 2.33 | 9.67 | 18.67 | 9.63 |
| 1.00 | 20.00 | 14.33 | 9.67 | 6.67 | 2.67 | 2.67 | 2.00 | 1.67 | 9.33 | 16.67 | 8.87a |
| 2.00 | 20.00 | 12.67 | 9.67 | 7.67 | 5.67 | 3.67 | 2.00 | 1.67 | 11.33 | 14.33 | 8.57 a |
| 4.00 | 20.00 | 11.00 | 4.67 | 4.00 | 3.33 | 2.67 | 0.00 | 0.00 | 0.00 | 0.00 | 4.57 |
| 8.00 | 20.00 | 10.33 | 4.00 | 2.67 | 1.67 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.87 |
| Control (Untreated grains) | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 22.00 | 25.00 | 38.00 | 22.50 |

CD ($p$=0.05) for Treatment=0.620; SEm± =0.221; CD ($p$=0.05) for Observation period=0.877; SEm±=0.312; CD ($p$=0.05) for Treatment×Observation period=1.960; SEm±=0.698; Values with the same superscript in a row/column do not differ significantly

### 3.3. Acaricidal activity of orange peel extract

Similar trend as observed in Aonla fruit and lemon peel extract was recorded with aqueous extract of orange peel also. The data in Table 4 revealed that a significantly lower number of live mites occurred in 0.5 (6.23 mites), 1 (5.37 mites), 2 (5.23 mites), 4 (4.00 mites) and 8% (3.57 mites) concentration of orange peelextract as compared to untreated grains(22.5 mites) (CD=0.305; $p$=0.05). *S. nesbitti* counts remained statistically comparable at 1 and 2% concentration.

Duration of treatment also significantly affected the efficacy of the treatment. The number decreased significantly from 20 mites at 0 day to 13.11, 9.22, 7.83, 6.00, 4.50, 3.67, 4.17 and 6.33 mites after 15 m, 30 m, 1 h, 2 h, 3 h, 1 day, 2 days, 7 days and 8 days of post treatment (CD=0.431; $p$=0.05). The number of mites recorded after 2 h and 15 days of treatment was at par with each other. Statistically comparable data for *S. nesbitti* count was recorded after 3h and 7 days, 1 and 2 days after orange peel extract treatment. The ANOVA showed significant difference in the number of mites in all the orange peel extract treatments at each observation period (CD=0.964; $p$=0.05) (Table 4). No mite was recorded in all the concentrations after 1 d of treatment.

### 3.4. Comparative evaluation of aqueous extracts of botanicals against suidasia nesbitti

During the present study, effect of aqueous extracts of botanicals against *Suidasia nesbitti* revealed that although Aonla fruit, lemon and orange peel possessed acaricidal activity but number of mites was statistically lower with orange peel (0 mites) treatment as compared to lemon peel (9.93 mites) and Aonla fruit (14.47 mites) treatment on pearl millet grains (CD=0.914; $p$=0.05) (Table 5).

Table 4: Effect of orange peel extract against *Suidasia nesbitti* in pearl millet grains

| Treatments (%) | Number of mites after treatment | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 m | 15 m | 30 m | 1 h | 2 h | 3 h | 1 day | 2 day | 7 day | 15 day | |
| 0.50 | 20.00 | 16.00 | 10.33 | 9.00 | 5.00 | 2.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.23 |
| 1.00 | 20.00 | 13.67 | 9.00 | 6.00 | 2.33 | 1.33 | 0.00 | 0.00 | 0.00 | 0.00 | 5.37 a |
| 2.00 | 20.00 | 11.00 | 8.67 | 6.67 | 5.00 | 2.33 | 0.00 | 0.00 | 0.00 | 0.00 | 5.23a |
| 4.00 | 20.00 | 9.00 | 4.00 | 3.00 | 2.67 | 1.33 | 0.00 | 0.00 | 0.00 | 0.00 | 4.00 |
| 8.00 | 20.00 | 9.00 | 3.33 | 2.33 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.57 |
| Control (Untreated grains) | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 22.00 | 25.00 | 38.00 | 22.50 |

CD ($p$=0.05) for Treatment=0.620; SEm±=0.221; CD ($p$=0.05) for Observation period=0.877; SEm±=0.312; CD($p$=0.05) for Treatment×Observation period=1.960; SEm±=0.698; Values with the same superscript in a row/column do not differ significantly

Table 5: Comparative efficacy of botanicals against Suidasia nesbitti in pearl millet grains

| Treatments (%) | Number of mites after 15 days of treatment with | | |
|---|---|---|---|
| | Aonla fruit extract | Lemon peel extract | Orange peel extract |
| 0.50 | 30.00 | 18.67 | 0.00 |
| 1.00 | 24.33 | 16.67 | 0.00 |
| 2.00 | 18.00 | 14.33 | 0.00 |
| 4.00 | 0.00 | 0.00 | 0.00 |
| 8.00 | 0.00 | 0.00 | 0.00 |
| Mean | 14.47 | 9.93 | 0.00 |

This showed orange peel extract was more potent than other two botanicals. Irrespective of botanicals, higher concentrations (8 and 4%) caused 100% mortality as no mites were recorded in these treatments after 15 days. The ANOVA revealed a significant interaction between treatments and botanicals (CD=2.045; $p$=0.05). It showed significantly less number of live mites at higher concentrations of all the three botanicals (0 mites) as compared to lower concentrations.In citrus and orange peels, d-Limonene is the main component which acts as repellent against pests (Klimek-Szczykutowicz et al., 2020). It was investigated against lesser grain borer, *Rhyzopertha dominica*; rice weevil, *Sitophilus oryzae*; and red flour beetle, *Tribolium castaneum* which showed ovicidal effects, oviposition-deterrent, development inhibition and feeding-deterrent activities (Ruiz-Pérez et al., 2016). The per cent of limonene is more in *C. sinesis* (orange) as compared to *C. limon* (lemon) (Klimek-Szczykutowicz et al., 2020) which may be the reason for strong acaricidal action of orange peel against *S. nesbitti* in the present study.

### *3.5. Determination of $LC_{50}$ values for suidasia nesbitti*

The present study showed thatorange peel extract caused 100% mortality in *S. nesbitti* population 1 d after the spray at all concentrations tested. Amongst two botanicals, aqueous extract of lemon peel extract was more effective against *S. nesbitti* than aqueous extract of Aonla fruit because of lower $LC_{50}$ value (Table 6). The $LC_{50}$ value was 0.64% in lemon peel extract while it was 1.38% in Aonla fruit extract against *S. nesbitti*. The value of slope (3.08 and 2.26) was less for both the extracts which showed that further increase in concentrations will lead to significant decrease in number of *S. nesbitti* mites.The intercept value was 4.74 and 5.24 for Aonla fruit and lemon peel extract, respectively.

Table 6: Determination of LC50 values of botanical aqueous extracts for Suidasia nesbitti

| Botanical extract | Direct spray bioassay (Leaf disc method) | | | | | |
|---|---|---|---|---|---|---|
| | n | Slope | Intercept | $LC_{50}$ (%) | $\chi^2$ | Df |
| Aonla fruit extract | 20 | 3.08 | 4.74 | 1.38 | 13.33 | 5 |
| Lemon peel extract | 20 | 2.26 | 5.24 | 0.64 | 15.21 | 5 |
| Orange peel extract | 20 | 100% mortality observed 1 d after the spray at all concentrations tested | | | | |

## 4. CONCLUSION

Pearl millet grains treated with aqueous extracts of Aonla fruits showed significantly lesser number of mites (4.67, 5.20 mites) at higher concentrations (8, 4%) as compared to lower concentrations. Lemon peel caused 100% mortality in *S. nesbitti* within 3 h of treatment at 8% and within 1 day at 4%. Among the three botanicals, orange peel extract is more toxicto *S. nesbitti* than the aonla fruit and lemon peel extract.

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