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Repellent Effects of Aqueous Extracts of Mustard seeds *Brassica juncea*, on three major pests of Horticultural Crops.

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Abstract:

Aqueous extract of mustard seeds was tested for its antifeedant activity against aphids (*Macrosiphum roseiformis*), thrips (*Scirtothrips dorsalis)* and spider mites, (*Tetranychus urticae)*. Choice-Nochoice bioassay experiment were planned against aphids, thrips, and spider mites, where two choices were given as food mustard seeds extract treated rose leaves and untreated (just water treated), to see if they had any preference. Aqueous extract of mustard seeds has antifeedant effect against all three pests studied, aphids, thrips, and spider mites. Based on these findings, mustard seed extract could be used in conjunction with modern, sustainable, and environmentally friendly pest management tactics in horticulture systems to combat these pests.

Key words: Mustard, *Macrosiphum roseiformis, Scirtothrips dorsalis, Tetranychus urticae,* Antifeedant

Indian mustard (*Brassica juncea*) seeds are the small round seeds 1 or 2 mm in diameter and varies in color from yellowish white to black. The mustard seeds contain 6.2g water, 24.6g protein, 35.5g fat, 28.4g total carbohydrate**,** 8.8g fiber and 5.3g ash [1]. Indian mustard is folk remedy for arthritis and foot ache [2]. In Korea, the seeds are used for abscesses, cold and stomach disorder [3]. Thirty aromatic medicinal plant species and five essential oils were tested against adults of *Sitophilus oryzae* and *Callosobruchus* sp. Among them *C. sieboldii*, *C. cassia* and *F. vulgare* as well as Cinnamon oil, horseradish oil and mustard oil were highly effective against these two insect pests [4].

Mustard had good inhibitory action against three potent food borne pathogens namely *Escherichia coli*, *Staphylococcus auresus* and *Bacillus cereus* at 1% concentration [5]. Volatile compounds released from tissue of two cover crop species - rye (*Cereale secale* L.) and

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oriental mustard (*Brassica juncea*) were tested on two important potato diseases *Rhizoctonia solani* and *Phythium ultimum*. At highest test dose, yield of potato increased by 54% and reduced the disease [6]. Three species of weed plants *Sonchus olearcues* L., *Brassica niger* Koch., *Raphanus sativa* var. surtus L. (hexane, petroleum ether and ethyl alcohol extracts) were tested against fourth instar larvae of Egyptian cottonworm (*Spodoptera littoralis*). The LC_{50} values of hexane extract of *S. olearcues*, petroleum ether extract of *B. niger* and ethyl alcohol extract of *R. sativa* were 218.36, 96.11 and 5574.66 ppm [7].

In order to prevent the negative effects of chemical pesticides on humans and nontarget organisms, future agriculture will require alternative biorational pest control strategies. Gupta et al. [8] demonstrated synergistic effects of biorationals when mixed with chemical insecticides for the control of thrips on roses. There is a plethora of research available that demonstrates how biologically produced insecticides offer enormous potential in pest management. *Carica papaya* aqueous leaf extract showed significantly higher values than control for percentage mortality (mortality range- 20%-70%) of aphids at all concentrations from X1 (0.25 g/ml) to X6 (0.0078125 g/ml) tested at 72 hr post treatment [9]. The extracts of fenugreek (*Trigonella foenum - graecum*), celery (*Apium graveolens*), radish (*Raphanus sativus*) and mustard (*Brassica compestris*) were tested against the third larval instar of *Cucila sericata* by ingestion method. The LC_{50} value of fenugreek, celery, radish and mustard were 2.81, 4.60, 6.93 and 7.92% [10]. Six plant extracts including kaner leaf, khejri leaf, neem leaf, safeda leaf, tomato leaf and mustard leaf and four plant powders. i.e black pepper powder, garlic clove powder, tulsi leaf powder and turmeric rhizome powder were tested against Callosoburchus *maculatus*. The maximum oviposition deterrence was observed with neem leaf and minimum with garlic clove powder [11]. Volatile compounds of mustard were detected by GC-MS and it was found that glucosinolates enzimatically decomposed compound was found in major proportion. [12]. The oriental (brown) mustard and yellow mustard extracts contained component: sinigrin, sinalbin, allyl isothiocynate and benzyl isothiocynate detected by reversed phase high performance liquid chromatography method [13]. The fumigant toxicity of synthetic mustard essential oil (SMEO) (90% allyl isothiocynate) was tested on the development stages of maize weevil (*Sitophilus zeamais*). The larvae were least tolerant [14]. The repellent activity of essential oils of camphor, castor, cinnamon, clove and mustard were tested against *Callosobrunchus maculatus*. All the essential oils with 1% concentration repelled the adult of *C. maculatus* except castor oil. Non-significant repellent effect was observed in cinnamon, clove and mustard [15]. The essential oil of camphor (*Eucalyptus globules*), castor *(Ricinus communis*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and black mustard (*Brassica rapa*) were used against *Bruchidius incarnates* (Coleoptera: Bruchidae). At 4% concentration essential oil of all plants (except castor oil) gave repellent effect [16].

MATERIALS AND METHODS

Rose (*Rosa* sp): - Ornamental rose plants are grown in the Rose garden of Panjab University Chandigarh, India. These constituted the experimental host plants for the present study.

Insect/mite pests: Rose aphids (*Macrosiphum roseiformis*), Thrips (*Scirtothrips dorsalis)* and Spider mites (*Tetranychus urticae)* were found infesting soft plant tissue of buds and fresh leaves. These parts were carefully cut and brought to the lab in plastic sample bags. The insect pests were released into petridishes by camel hairbrush (1 mm size) for experimentation.

Natural Plant material:

Mustard *(Brassica juncea)* is a source of leafy vegetable and seeds used as spice and for producing oil. These seeds were obtained from the market. The samples of these plant materials were sent to the Department of Botany, Panjab University, Chandigarh, India, for identification and a catalogue number (7557 mustard) was obtained.

Plants extract preparation: Mustard seeds were washed with water only. Mustard seeds were air dried for some time to get rid of extra water. 100gm of each material was taken and ground in 250ml of water in mixer-grinder slowly for 5 minutes. It was then sieved through muslin cloth. The prepared extract was kept in refrigerator for further use only up to seven days and after that it was discarded. Fresh extracts were made for further experimentation.

Food choice- no- choice bioassays for Aphids, Thrips and Spider mites: Choice and no-choice assay methods were followed according to Gupta [17]. Mustard seeds extract treated rose leaves and untreated (only water treated) rose leaves were offered as food source to the test aphids, thrips and spider mites in separate bioassay experiment in order to observe the preference if any exercised by them. Observations on number of insects/mites present on treated or untreated rose leaves were made at 30, 90 and 180 min. All the replicates were combined and analyzed by χ^2 goodness of fit test [18].

It was observed that there were no aphids on mustard seeds extract treated rose leaves at 30 minute (6.7%) while 6.7% were found on untreated leaves (χ^2 = 1.59, P = 0.2077). The average number of thrips on mustard seeds extract treated rose leaves at 30 min after start of treatment was 0% and it was 20% on untreated leaves (χ^2 = 3.82, P = 0.0507). There were no spider mites on mustard seeds extract treated rose leaves at 30 min (0%). There were 26.7% on untreated leaves (χ^2 = 3.82, P = 0.0507). The result is represented in Figure 1.

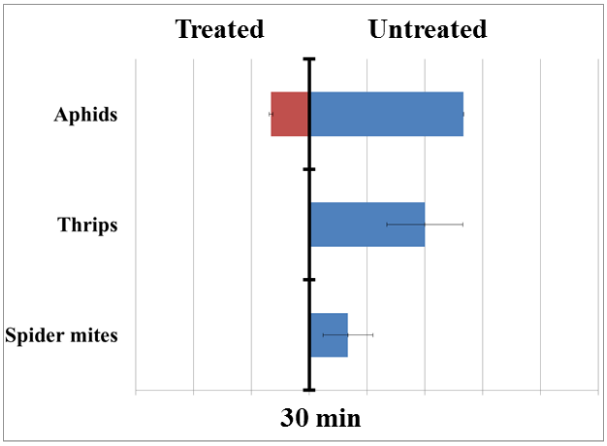

Figure 1: Average percentage of aphids, thrips and spider mites on treated and untreated leaves of rose at 30 min after treatment.

There were still no aphids on mustard seeds extract treated rose leaves at 90 minute (0%) however on untreated rose leaves the number increased to 33.3% (χ^2 = 8.32, P = 0.0039) There were no thrips at 90 minute on mustard seeds extract treated rose leaves at 90 min (0%). On untreated leaves thrips were observed 46.7%. (χ^2= 8.32, P = 0.0039). The same result was observed after 90 min i.e 0% on treated rose leaves. There were 46.7% on untreated leaves (χ^2 = 8.32, P = 0.0039). This data is presented in Figure 2**.**

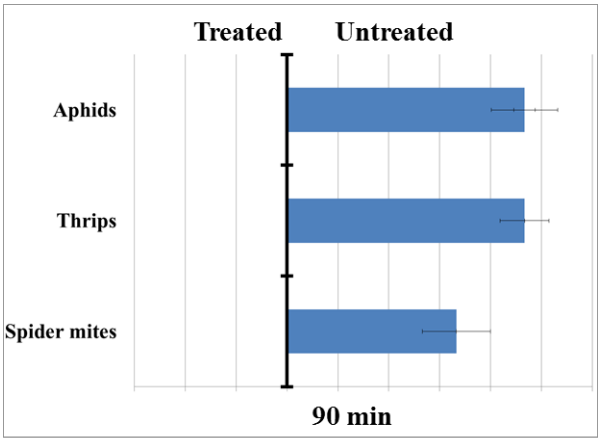

Figure 2: Average percentage of aphids, thrips and spider mites on treated and untreated leaves of rose at 90 min after treatment.

After 180 minutes of release, it was observed that there were 6.7% and 60% aphids on treated and untreated leaves respectively (χ^2 =1.53, P = 0.2168). Observation revealed that there were no thrips on mustard seeds extract treated rose leaves at 180 min (0%). On untreated leaves thrips were observed 53.3% (χ^2= 8.32, P = 0.0039**)**. Average number of spider mites on mustard seeds extract treated rose leaves at 180 min was 6.7%. It was 46.7% on untreated leaves (χ^2 = 8.32, P = 0.0039**).** This data is presented in Figure 3.

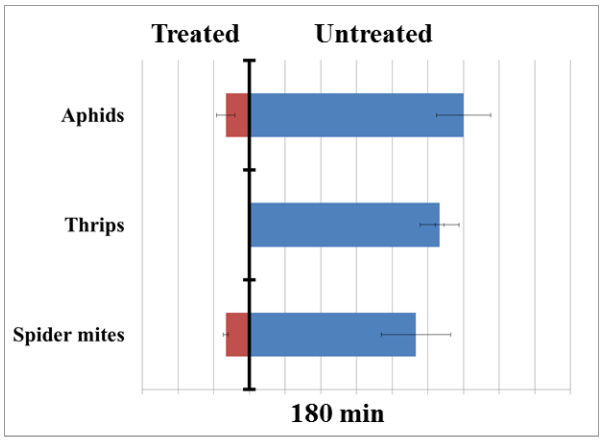

Figure 3: Average percentage of aphids, thrips and spider mites on treated and untreated leaves of rose at 180 min after treatment.

DISCUSSION

Mustard seeds are the small round seeds 1 or 2 mm in diameter and yellowish white to black in color. The mustard seeds contain 6.2g water, 24.6g protein, 35.5g fat, 28.4g total carbohydrate**,** 8.8g fiber and 5.3g ash. Indian mustard is folk remedy for arthritis and foot ache. In Korea, the seeds are used for abscesses, cold and stomach disorder. Besides they have insecticidal effects for stored grain pests. In present study the repellent effect of mustard seed aqueous extract has been investigated against three important sucking pests of horticultural crops. Results are discussed as following.

Choice- no- choice assays against aphids revealed that when two choices as mustard seeds extracts treated and untreated leaves were given to the aphids, at 30, 90 and 180 minutes observation, they showed preference towards untreated leaves. Results indicated that mustard seeds had repellent effect towards aphids. Similar repellent effects were noticed with other biorational compounds in our earlier studies against aphids viz: Cow's milk [19], wild pomegranate peel and seeds aqueous extracts [20], Citrus peel aqueous extracts [21] and also with Pomegranate fruit peel aqueous extracts [22]. Other studies also demonstrated efficacies of different biologically obtained compounds as **Santos *et al.*** [23] evaluated the effects of aqueous extracts of neem (*Azadirachta indica* A. Juss) seed powder on the development, survival and fecundity of *A. gossypii*. They concluded that the aqueous extract of neem seeds was efficient against the aphid *A. gossypii*, causing nymph mortality and reducing survival period and fecundity. The toxicity of oil extracts of five medicinal plants (*Achillea santolina*, *Artemisia seiberi*, *Salvia officinalis*, *Thymus vulgaris* and *Senecio vernalis*) was evaluated against the woolly apple aphid, *Eriosoma lanigerum.* Results revealed that LC_{50} at 24 hr post treatment is 7070, 6161, 6667, 19090 and 9034 ppm for *A. santolina*, *A. seiberi*, *S. officinalis*, *T. Vulgaris* and *S. vernalis*, respectively. *A. seiberi* oil extract was the most toxic to woolly apple aphid in terms of dose and time responses compared with other tested oil extracts [24]. The effect of essential oils from five medicinal plants including *Zataria multiflora* and *Nepeta cataria* (Lamiaceae), *Tagetes minuta* and *Artemisia siberi* (Asteraceae), and *Trachyspermum ammi* (Apiaceae) were analyzed against the adult stage of the cabbage aphid, *Brevicoryne brassicae* L. The essential oils of *N. cataria* at 126, 63, 31, 16, 8, and 3 µl L-1 air caused 94, 76, 52, 46, 36, and 24% mortality within 24 hours, respectively [25]. Ethanol and water extracts of five medicinal and ornamental plant species namely, *Aerva lanata*, *Ruta chalepensis, Fagonia arabica, Malva parviflora* and *Calotropis procera* were evaluated against pomegranate aphid, *Aphis punicae Passerini* under laboratory condition. Results indicated that the ethanol extract of *R. chalepensis* (whole plant) showed the highest repellency (75%) and mortality (79.5%) at 0.015% concentration. On the other side, water extracts of *A. lanata* caused the highest repellency (44.88%) and mortality (61.2%) at 5% concentration [26].

Choice- no- choice data against thrips revealed that when two choices as mustard seeds extract treated and untreated leaves were given to the thrips, at 30, 90 and 180 minutes observation, they showed preference towards untreated leaves. Results indicated that mustard seeds had repellent effect towards thrips (Figure 2). As this extract had repellency towards thrips the reason for their mortality at 48 hrs is assumed to be because of starvation and not due to toxicity. The insecticidal activity of aqueous spice extracts was studied on pest insects (*Bemisia tabaci*, *Caliothrips impurus*, *Caliothrips occipitalis* and *Nisotra spp*.) found on green beans. These spices contained chemicals (piperidine alkaloids, saponosides, anthraquinones and triterpenes), which had

insecticidal properties. The differents extracts applied at the dose of 400 l/ha (100 g powder/L l water) reduced total infestation populations of whitefly by 61-76%, thrips by 67-75% and coleoptera by 72- 78%, compared to the untreated control [27]. The bio-efficacy of *Calotropis gigantea* (L), *Annona squamosa* (L), *Leucas aspera* (Willd.), *Zingiber officinale* (Roscoe), *Curcuma longa* (L), *Allium sativum* (L), *Capsicum annuum* (L) and *Acorus calamus* (L) with cow urine was found to control *Pseudodendrothrips mori* (Niwa). On 26th day of treatment, the amalgamated plant extract and dichlorvos reduced the pest up to 95.6% and 61.14%, respectively [28].

Spider mites with choice- no- choice data revealed that when two choices as mustard seeds extract treated and untreated leaves were given as food, at 30, 90 and 180 minutes observation, the spider mites showed preference towards untreated leaves. Results indicated that mustard seeds had repellent effect towards spider mites. Mustard showed insecticidal activities against various pests. Thirty aromatic medicinal plant species and five essential oils were tested against adults of *Sitophilus oryzae* and *Callosobruchus* sp. Cinnamon oil, horseradish oil and mustard oil were highly effective against these two insect pests [4]. Three species of weed plants *Sonchus olearcues* L., *Brassica niger* Koch., *Raphanus sativa* var. surtus L. (hexane, petroleum ether and ethyl alcohol extracts) were tested against fourth instar larvae of Egyptian cottonworm (*Spodoptera littoralis*). The LC_{50} values of hexane extract of *S. olearcues*, petroleum ether extract of *B. niger* and ethyl alcohol extract of *R. sativa* were 218.36, 96.11 and 5574.66 ppm [7]. The extracts of fenugreek (*Trigonella foenum - graecum*), celery (*Apium graveolens*), radish (*Raphanus sativus*) and mustard (*Brassica compestris*) were tested against the third larval instar of *Cucila sericata* by ingestion method. The LC_{50} value of fenugreek, celery, radish and mustard were 2.81, 4.60, 6.93 and 7.92%. Treatment of larvae with mustard (8%), radish (12%) and fenugreek and celery (16%) suppressed adult emergence. Morphological abnormalities of larvae, pupae and adult were also recorded [10]. Six plant extracts including kaner leaf, khejri leaf, neem leaf, safeda leaf, tomato leaf and mustard leaf and four plant powders i.e. black pepper powder, garlic clove powder, tulsi leaf powder and turmeric rhizome powder were tested against *Callosoburchus maculatus*. The maximum oviposition deterrence was observed with neem leaf and minimum with garlic clove powder [11]. Han *et al.* [29] evaluated three different medicinal plants (*Mentha longifolia*, *Salvia officialis* (both Lamiaceae) and *Myrtus communis* (Myrtaceae)), against *T. urticae*. Results obtained indicated that *M. longifolia* possesses the highest lethal activity whereas *S. officialis* the lowest. Also, essential oils of *M. longifolia*, *M. communis*, and *S. officialis* were demonstrated to possess repellency effect. The fumigant toxicity of synthetic mustard essential oil (SMEO) (90% allyl isothiocynate) were tested on the development stages of maize weevil (*Sitophilus zeamais*). The larvae were least tolerant [14].

CONCLUSION

In conclusion, the aqueous extract of mustard seeds had antifeedant efficacy against all three pets tested: aphids, thrips, and spider mites. On the basis of these findings, it is suggested that mustard seed extract be used in conjunction with modern, sustainable, and environmentally friendly pest management practices in horticulture systems to combat these pests.

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